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Synthesis of macromolecular systems via lipase catalyzed biocatalytic reactions: applications and future perspectives†

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Enzymes, being remarkable catalysts, are capable of accepting a wide range of complex molecules as substrates and catalyze a variety of reactions with a high degree of chemo-, stereo- and regioselectivity in most of the reactions. Biocatalysis can be used in both simple and complex chemical transformations without the need for tedious protection and deprotection chemistry that is very common in traditional organic synthesis. This current review highlights the applicability of one class of biocatalysts viz. “lipases” in synthetic transformations, the resolution of pharmaceutically important small molecules including polyphenols, amides, nucleosides and their precursors, the development of macromolecular systems (and their applications as drug/gene carriers), flame retardants, polymeric antioxidants and nanocrystalline solar cells, etc.

1. Introduction

Enzymes are catalysts evolved by nature to sustain biochemical reactions in a living cell. Metabolic pathways and cell growth require highly diverse reactions that are catalysed by a wide variety of natural enzymes. As a consequence, enzymes have evolved as highly specialised catalysts for different types of chemical transformations. However, the synthetic potential of enzymes was unknown until 1897, when Eduard Buchner's work proved that enzymes do not require the environment of a living cell to be active. This discovery led to the application of enzymes in various fields, one being the production of chemicals via biotransformations. In the 1980s the scope of biocatalysis was expanded for new applications in synthetic chemistry by the observation that enzymes are active in organic solvents containing little or no water.^{1,2}

Among the various classes of enzymes known, lipases (hydrolytic enzymes) have attracted immense interest in recent years. Lipases (EC 3.1.1.3) are ubiquitous enzymes found in all types of living organisms from fungi and bacteria to plants and animals. Lipases exert their natural function towards the hydrolysis/synthesis of carboxylic ester bonds of long-chain triacylglycerols. Beside their role in lipid metabolism, lipases are widely used in biotechnology as additives in detergents, as food ingredients, as pitch control in the pulp and paper industry, and in biopolymers and biodiesel production, etc.³ The properties of lipases, such as their stability in organic

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† With pleasure, we dedicate this review article to the memory of our great friend and collaborator, (Late) Professor Sukant Tripathy (Professor of Chemistry, Founder Director of the Centre for Advanced Materials and Provost of the University of Massachusetts Lowell, UML, USA) who was responsible for the initiation of the highly successful and productive collaboration between the University of Delhi (DU, India) and the University of Massachusetts Lowell (UML, USA) that has sustained for 20 long years, and has benefited a very large number of students and staff of DU during their short and long stays at the UML.

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solvents, catalytic activity without cofactors, broad substrate specificity, and high reaction chemo-, regio- and stereo-selectivity make them the most versatile class of enzymes in synthetic organic chemistry.^{4,5} Ongoing efforts from our research laboratories, over the last three decades, involving lipase-mediated biocatalysis have proved to be exceedingly useful in developing highly selective and 'greener processes' for pharmaceutical, fine and specialty chemical industries (Fig. 1).⁶ We have explored a number of lipases to synthesise enantiomerically pure pharmaceutical key intermediates

and macromolecular architectures with an aim towards broad and diverse applications as summarized in this review.

2. Biocatalytic approach to small molecules

The synthesis of medicinally significant low molecular weight scaffolds which are either naturally occurring or inspired by nature, involving methodologies to achieve the desired target



Dr Amit Kumar received his BSc (2006), MSc (2008) and PhD (2014) in Chemistry from the University of Delhi (India). Prior to his PhD, he was a research associate (2008–2010) at Jubilant Chemsys Ltd, Noida (India). He has worked as a visiting scholar at the University of Leuven (KU Leuven), Belgium, during Dec 2011–March 2013. Currently, he is a Dr Kothari Post-doctoral Fellow at Jamia Hamdard University, New Delhi (India). His

research focuses on the biocatalytic synthesis of PEG-based polymers and the development of new protocols for the synthesis of heterocyclic frameworks via post-multicomponent reactions using transition metal catalysis.



Dr Shashwat Malhotra received his Master's in Organic Chemistry in 2004 from Banaras Hindu University, India. He completed his PhD in 2010 in Organic Chemistry from the University of Delhi (India). In 2010, he joined the research group of Prof. Dr Rainer Haag at the Freie Universitat Berlin for his postdoctoral work. Presently, he is an Assistant Professor at Kirorimal College, University of Delhi (India). His research interests are dedicated towards the development of novel

methodologies, and delivery systems based on multifunctional dendritic architectures and self-assembling amphiphiles for the controlled delivery of bioactives.



Dr Mukesh K. Pandey obtained his BSc (Honors) and MSc degrees in Chemistry with a specialization in Medicinal Chemistry from Banaras Hindu University, India. He obtained his PhD from the University of Delhi, India. Dr Pandey joined the laboratory of Professor A. C. Watterson for his postdoctoral training in enzyme catalyzed polymer synthesis and later moved to Harvard Medical School, Boston, to receive advanced training in Radiochemistry and

Molecular Imaging. Since 2012, Dr Pandey has been serving as an Assistant Professor of Radiology at Mayo Clinic Rochester. Dr Pandey is one of the recipients of the Mayo Clinic Radiology Advance Award. His research interests are in the design and development of positron emission tomography imaging probes for the imaging and diagnosis of neurodegenerative and psychiatric disorders. He also has interests in the development of novel polymeric drug delivery approaches to improve the pharmacokinetics and pharmacodynamics of natural products.



Prof. Dr Rainer Haag obtained his PhD with Prof. Dr A. de Meijere at the University of Go'ttingen in 1995. After postdoctoral work with Professor S. V. Ley, University of Cambridge (UK), and Professor G. M. Whitesides, Harvard University, Cambridge (USA), he completed his habilitation at the University of Freiburg in 2002. He then became Associate Professor at the University of Dortmund and in 2004 was appointed full Professor of Organic and Macromolecular

Chemistry at the Freie Universita't Berlin. His main research interests are the mimicry of biological systems by functional dendritic polymers, with particular focus on applications in nanomedicine.

molecule in an efficient and cost effective manner is of prime concern for chemists.⁷ Though chemocatalysis and biocatalysis continue to be active areas of academic research and business investment,⁸ the goal of biocatalysis is to steer the reaction efficiency in comparison to the earlier reported approaches to achieve low molecular weight scaffolds. Our efforts led to the development of a toolbox of facile chemoenzymatic approaches for the synthesis of targeted small molecules of interest using lipases. The details of this approach are discussed below.

2.1. Polyphenolics

Polyphenols are known to be beneficial in a number of diseases including cardiovascular or neurodegenerative diseases, osteoporosis and cancer.⁹ Suitably substituted polyhydric phenols are also used as starting materials for the synthesis of different classes of natural polyphenolics viz. chalcones, flavones, flavanones, isoflavonoids, coumarins, xanthenes, and catechins, etc. Selective protection and deprotection steps are often required to achieve the total synthesis of these compounds, which are rather difficult. Moreover they increase the number of steps and decrease the net yields thus making the overall synthesis quite cumbersome. It was therefore envisioned to employ enzymes when carrying out selective transformations and thus avoiding the cumbersome protection/deprotection synthetic steps to achieve the desired intermediates. Over the years, we have established the use of lipases from porcine pancreas (PPL), *Candida rugosa* (CRL), *Candida antarctica* (CAL), *Candida cylindracea* (CCL), *Aspergillus terreus* (ATL), *Aspergillus carneus* (ACL), and from *Fusarium* and *Pseudomonas* species when carrying out selective

transformations on various peracetylated phenolic compounds representing various classes of natural products e.g. flavones, flavanones, coumarins, ketones, chromanones, diphenyl ethenes, chalcones, catechins, etc. and their intermediates (Fig. 2).^{10–19}

Selective deacetylations were studied on these substrates and it was observed that while using porcine pancreatic lipase (PPL), selective deacetylation occurred at the para- and meta-positions with reference to the nuclear carbonyl group, in comparison to that at the ortho-acetoxy group. These results were found to be important and interesting as an ortho-acetoxy moiety would preferentially undergo deacetylation under chemical conditions due to chelation of the resulting hydroxyl group with the carbonyl group. We proposed that the selective transformation occurs via the formation of a dynamic Schiff's base enzyme–substrate complex between the nuclear carbonyl group of the substrate and the amino group of lysine of the enzyme, which is present in the active site of PPL. To confirm this hypothesis of enzymatic deacylation, a study was conducted with a number of variously substituted acetoxy aromatic amides and peracetates.^{20,21} It was concluded that this strategy of acetylation can also be employed in the enantiomeric resolution of racemic ketones,²² which are important precursors in the synthesis of biologically active chromones, amides and isoflavanones.^{23–28}

2.2. Biocatalytic reactions in nucleoside chemistry

Nucleosides are fundamental building blocks of biological systems and show a wide range of biological activities. They are sequentially phosphorylated by kinases into their mono-, di- and triphosphates and the resultant nucleotides are processed



Arthur C. Watterson

Professor Arthur C. Watterson is a Professor Emeritus at the Department of Chemistry, University of Massachusetts, Lowell, USA (UML). He received his BS degree from Geneva College (1960) and PhD from Brown University (1965). He has two years of post-doctoral experience at Johns Hopkins University (1964–1965). He then joined the UML faculty and served as Dean (1986–1992), and Chairman of Chemistry Department (1984–1986). Also,

he has been the 'Director' of Institute of Nano Science Engineering and Technology (INSET) since 2001. His research areas include Biocatalysis, Materials Science and Polymer Science. He has published 175 research articles and has five US patents awarded.



Professor Virinder S. Parmar obtained his BSc Honors, MSc and PhD from the University of Delhi, and did his postdoctoral research at Cornell University, Harvard University, the University of Basel and Imperial College. He has been a faculty member at the University of Delhi for 44 years and has served as Head of the Department of Chemistry and Chairman of Board of Research Studies. He has been a Visiting Professor at

the University of Massachusetts, University of Southern Denmark, Institute of Advanced Sciences and the Central University of Haryana. His research interests include: Nanoscience, Synthetic Organic Chemistry, Biocatalysis, Polymer Chemistry, Nucleic Acid Chemistry, Green Chemistry, Advanced Materials and Chemistry of Natural Products. He has mentored 85 PhD and Postdoctoral Scientists, and has published 476 research papers in addition to being co-inventor on 11 patents. He is a member of the IUPAC Organic and Biomolecular Chemistry Division's Subcommittees on Biomolecular Chemistry and Green Chemistry.

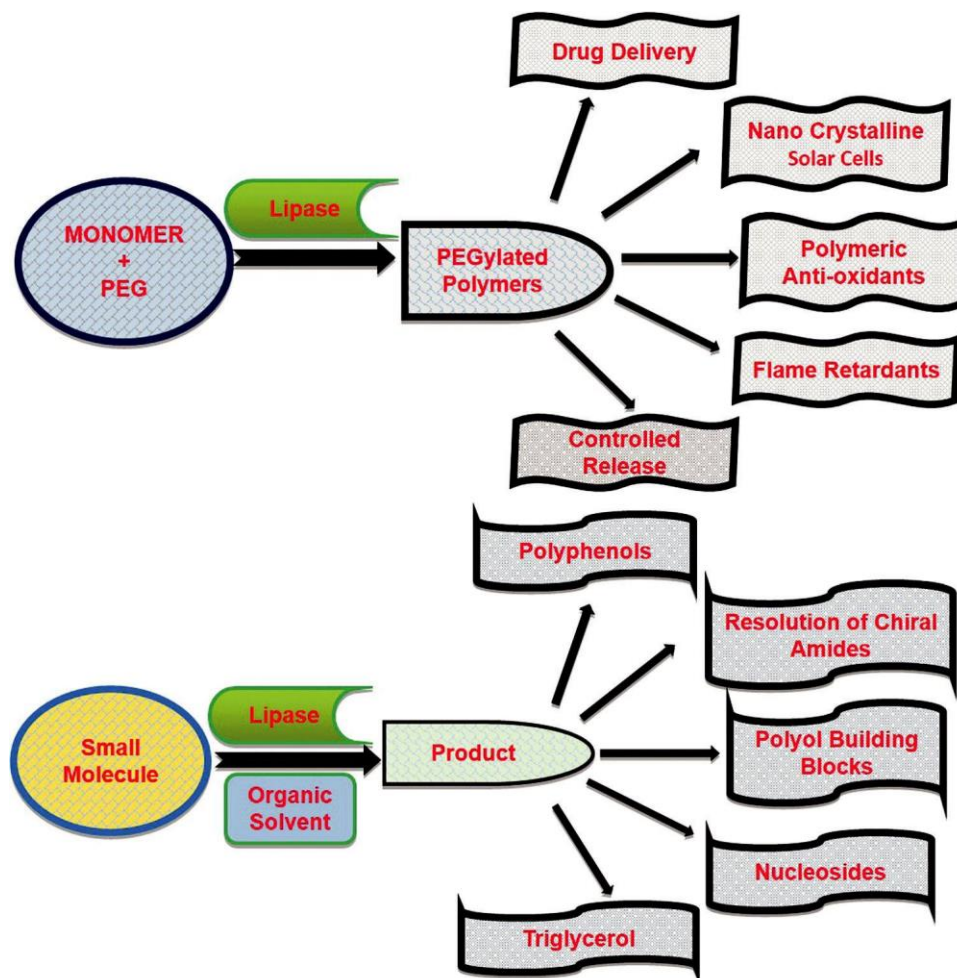


Fig. 1 Schematic presentation of the lipase catalysed formation of small and polymeric materials and their applications.

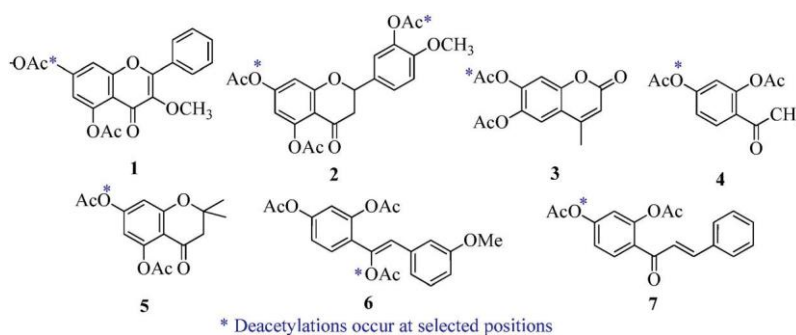
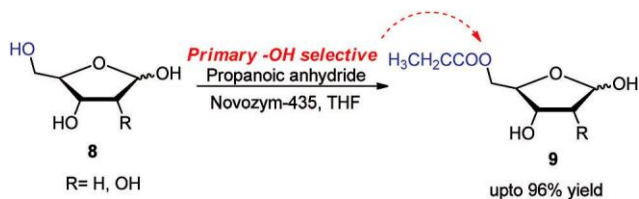


Fig. 2 Lipase catalysed selective deacetylation reactions.

into nucleic acids by polymerases.^{29,30} The search for clinically useful nucleoside analogues and derivatives has resulted in a wealth of new approaches for their synthesis, the prominent ones in recent years have been through enzymatic catalysis. Biocatalytic reactions are becoming standard procedures in their synthesis due to their feasibility and efficiency.^{31,32} In a similar endeavour, the regioselective acylation of the primary hydroxyl group of pentoses **8** with propanoic anhydride (Scheme 1) in THF was carried out using Novozym 435 as a catalyst affording exclusively

the monoacylated derivative **9** in 95–96% yields.³³ This method could be useful for the highly selective monoprotection of the primary hydroxyl group of a large variety of carbohydrate derivatives.^{34,35}

The regioselective acylation of secondary hydroxyl groups of deoxyribo- and ribonucleosides using lipases, e.g. *Pseudomonas cepacea* lipase (PSL) and lipase KWI-56 (a lipase from *Pseudomonas* sp.) are also reported in the literature.^{36–38} Similarly, we have also explored the use of lipases for the regioselective acylation of



Scheme 1 Lipase catalysed regioselective primary –OH group acylation of pentoses.

secondary hydroxyl groups of deoxyribo-, arabino- and ribonucleosides (10, 12 and 14) by a novel bacterial lipase, i.e. *Pseudomonas aeruginosa* lipase (PAL) using butanoic anhydride as an acylating agent affording exclusively the monoacylated derivatives of 11, 13 and 15, respectively (Scheme 2).³⁹

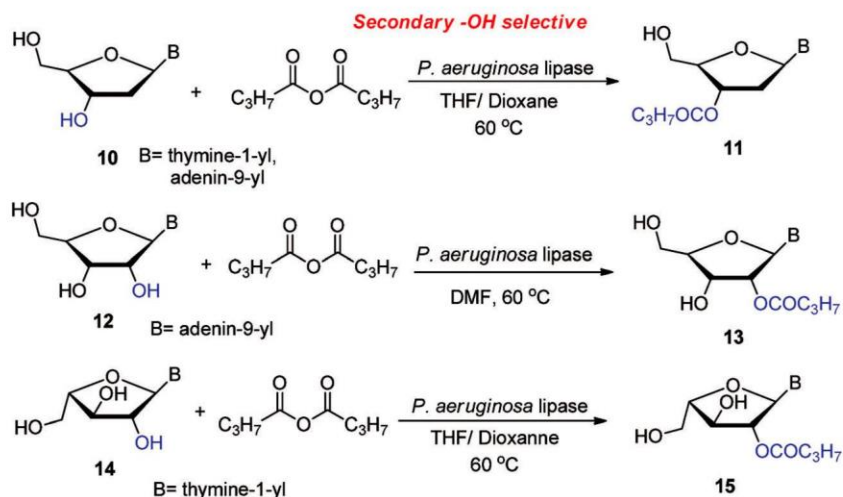
Unlocked nucleic acid (UNA) has the ribose ring open between the 2'- and 3'-carbon atoms and is an acyclic analogue of RNA. Modification of siRNA with UNA nucleotides has shown highly potent gene silencing activity accompanied by low cell toxicity.^{40,41} For selective benzoylation of the 2'-hydroxyl function in 5'-O-DMT-2',3'-secouridine 17 with vinyl benzoate as an acyl donor, several lipases were screened in different organic solvents. It was demonstrated that the Lipozyme TL IM in toluene can effectively discriminate between the two primary

hydroxyl groups present at the 2' and 3' positions in 5'-O-DMT-2',3'-secouridine 17. This progress allowed for the development of an environmentally friendly and highly selective methodology for the efficient synthesis of 2'-O-benzoyl-5'-O-DMT-2',3'-secouridine 18 in almost quantitative yields (Scheme 3). Compound 18, obtained by enzymatic benzoylation, was subsequently used for the synthesis of 3'-O-phosphoramidite 19 in a satisfactory yield, which is a building block for the preparation of oligonucleotides containing the uracil monomer of UNA.⁴²

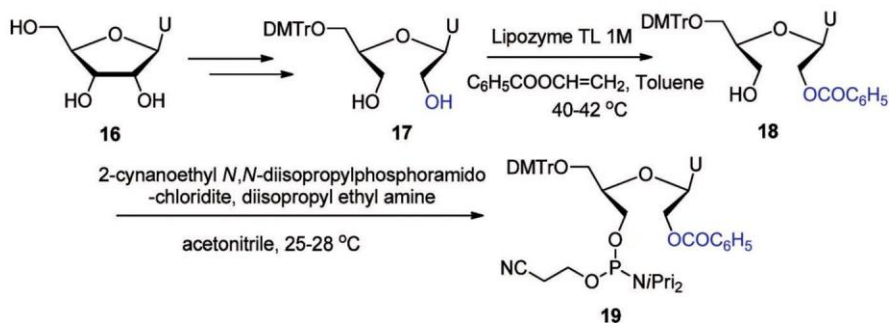
The anomeric separation of O-aryl α,β-D-ribofuranosides is not feasible by conventional chromatographic methods. To overcome this issue, an efficient and useful lipase-based method was developed to separate the α- and β-anomers using one of the acetoxy functions of peracetylated O-aryl α-D-ribofuranoside as a handle (Scheme 4). This methodology facilitates the easy synthesis of both, α- and β-anomers of O-aryl D-ribofuranosides, which showed mild to medium antitumor activities.⁴³

2.3. Biocatalytic approach to polyol building blocks

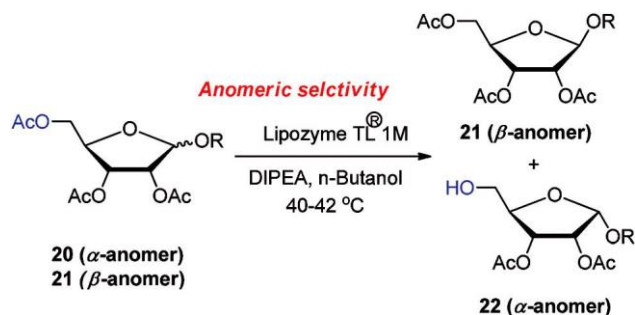
Polyols are polyhydroxy compounds and are important building blocks of polyurethanes and polyesters that are useful in a wide range of applications such as construction materials, coatings agents, adhesives, sealants, elastomers, and resins, etc. Enantio-selective transformations of these polyhydroxy compounds is



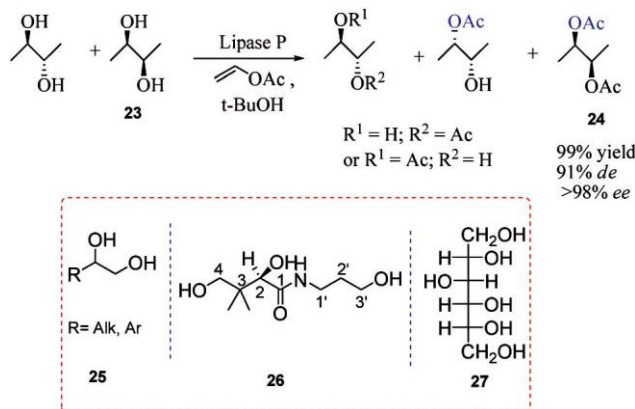
Scheme 2 Lipase catalysed regioselective acylation of secondary –OH groups of deoxyribo-, arabino- and ribonucleosides.



Scheme 3 Lipase catalysed regioselective acylation of UNA.



Scheme 4 Lipase catalysed anomeric resolution of O-aryl α,β -D-ribofuranosides.



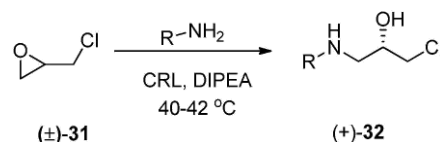
Scheme 5 Lipase catalysed enantioselective acylation of butane-2,3-diol.

important considering the useful materials synthesized from these intermediates. An extraordinary selectivity of the lipase, Amano P to convert the stereoisomeric mixture, () and meso 2,3-butane diol (23) into its (2R,3R)-diacetate 24 with 91% de and 498% ee on esterification with vinyl acetate (Scheme 5) shows the promising aspect of enantioselectivity through biocatalysis.⁴⁴

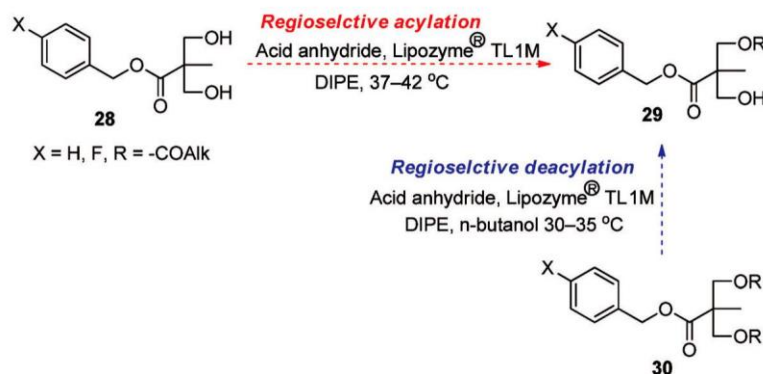
It has been observed that PPL and CRL allow differentiation of primary and secondary hydroxyl groups. We have studied the regioselective transesterification of 1,2-diols 25 with the acylating agent 2,2,2-trifluoroethyl butyrate (TFEB) by using PPL and CRL. The reaction proceeds with high regioselectivity,

and acylation takes place preferentially at the primary hydroxyl groups over the secondary groups.⁴⁵ In the case of compound 26, which has two primary and one secondary hydroxyl groups, only the C-3⁰ primary hydroxyl group at the far end position to the asymmetric carbon gets acylated. These results are in conformity with the active site model of the lipase proposed by Bhalerao et al.⁴⁶ Exploring these reactions, a very high yield of sorbitol-monostearate was obtained through A. terreus lipase (ATL) catalysed esterification of sorbitol (27) with stearic acid. This involved the selective esterification at one of the two primary hydroxyl groups without any reaction at any of the four secondary hydroxyl groups.⁴⁷ In our recent work, another biocatalytic regioselective acylation of the primary hydroxyl group in diols 28 and deacylation of their diesters 30 in the presence of Lipzyme^s TL 1M in diisopropyl ether is reported (Scheme 6).⁴⁸ Both the acylation and deacylation reactions were highly selective and efficient, yielding exclusively the monoacylated products 29 in very good yields. The biocatalytic route developed in these studies clearly demonstrates the superiority of enzymatic selective manipulation of prochiral diols and polyhydroxy compounds, the partial esters obtained in these studies can serve as useful monomers for making amphiphilic copolymers, which have potential utility in the health and industrial sectors.

In another work, regio- and enantioselective synthesis of (S)-(+)-3-arylamino-1-chloropropan-2-ols 32 has been achieved by the selective epoxide ring opening of ()-epichlorohydrin 31 with different aromatic amines in the presence of Candida rugosa lipase (Scheme 7).⁴⁹ Seven model (S)-(+)-3-arylamino-1-chloropropan-2-ols (+)-32 have been evaluated for the inhibition of tumor necrosis factor- α (TNF- α) induced expression of intercellular adhesion molecule-1 (ICAM-1), and have been found to exhibit up to 86% inhibition of TNF- α induced



Scheme 7 Lipase catalysed enantioselective synthesis of (S)-(+)-3-arylamino-1-chloropropan-2-ols.



Scheme 6 Lipzyme^s TL 1M regioselective acylation and deacylation.

expression of ICAM-1 at a concentration of 40 mg mL⁻¹, thus showing promising anti-inflammatory activity.

3. Biocatalytic regioselective transesterification of polyglycerol

Dendritic polymeric architectures derived from polyglycerol (PG) have been extensively used in the design of scaffolds in several biomedical applications.^{50–52} Due to their highly biocompatible nature, dendritic PGs (dPGs) show a broad range of potential applications in medicine and pharmacology, as well as the release of bioactive molecules in regenerative medicine in the form of non-fouling surfaces and matrix materials. The presence of amine groups on the surface or focal point in polyglycerol (PG) architectures has proved to be useful for providing an anchoring point in the preparation of protein resistant surfaces,^{53,54} in the synthesis of polyglycerol (PG)-based peptide conjugates,⁵⁵ macromolecular prodrugs (compounds that, after administration in the body, are converted into a pharmacologically active drug) and in the condensation of nucleic acid drugs.^{56–58} The amount and localization of amine groups within the PG scaffold have shown to have an effect on several properties related to drug coupling efficiency, cellular uptake, gene transfection efficiency,⁵⁹ toxicity and non-specific interactions (e.g. to albumin).⁶⁰ To better tune these properties of amino-polyglycerol compounds, it is desirable to develop a synthetic methodology which allows control of the loading and localization of the amine moieties. We have developed a highly efficient temperature-dependent chemo-enzymatic methodology for the regioselective synthesis of novel esters of glycerol, i.e. G1 tri-glycerol dendrons and related esters using 4-nitrophenyl 2-(tert-butoxycarbonyl)acetate (Boc-gly-Ph-pNO₂, 33) as the acylating agent for the first time. This methodology offers efficient and controlled loading of amino acid (glycine) on polyhydroxy compounds. Novozym 435, porcine pancreatic lipase (PPL), *Candida rugosa* lipase (CRL), *Thermomyces lanuginosus* lipase immobilized on silica (Lipozyme TL IM) and *Candida antarctica* lipase-B were analysed for selective transesterification reactions on glycerol. Using Lipozyme TL IM in dioxane and N-Boc-glycine 4-nitro-phenyl ester 33 as the acylating reagent for the selective protection of hydroxyl groups of glycerol, different products were formed at two different temperatures (50 °C and 70 °C). Regioselective transesterification on glycerol under these conditions facilitated the formation of mono- and diesterified products, which were used to furnish the bifunctional G1 glycerol dendrons. Compounds 34 and 35 bearing four hydroxyl groups (two primary and two secondary hydroxyl groups) on Lipozyme-catalyzed biocatalytic transesterification reactions using N-Boc-glycine 4-nitrophenyl ester (33) at 50 °C for 30 h in dioxane, afforded compounds 36 and 37 in 81% and 76% isolated yields, respectively, in which only one primary hydroxyl group in both the cases was esterified (Scheme 8).⁶¹ The mono esters 36 and 37, when further subjected to Lipozyme-catalyzed transesterification reaction with N-Boc-glycine 4-nitrophenyl ester (33) for another 30 h at 50 °C in dioxane, gave compounds 38 and 39 (with both the primary

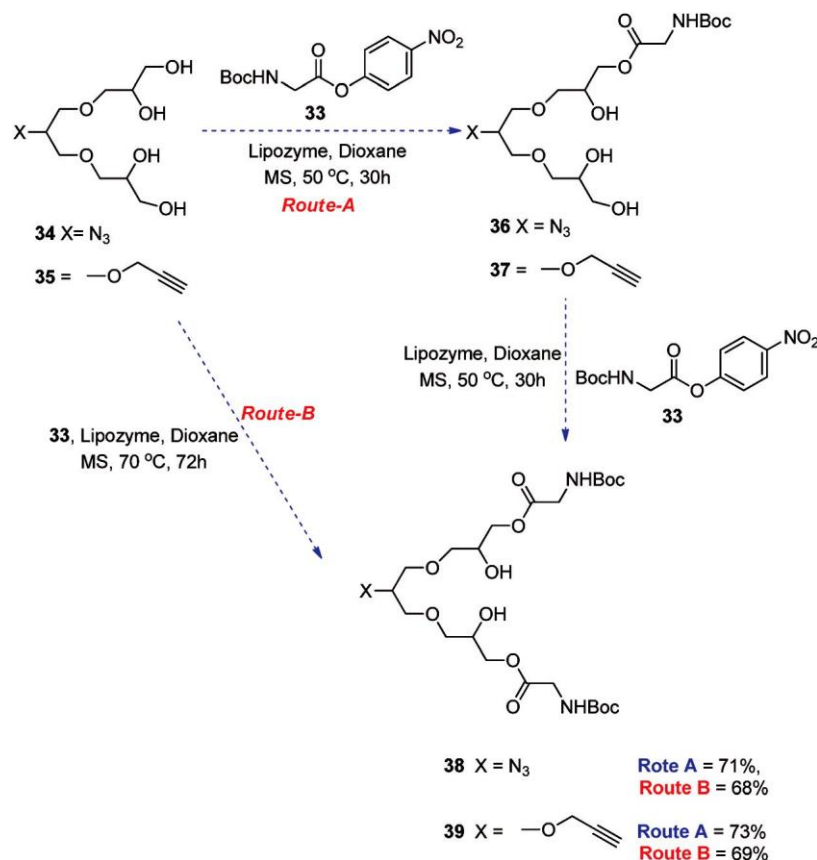
hydroxyl groups esterified) in 71% and 73% yields, respectively. The same transesterification reaction, when carried out on compounds 34 and 35 at 70 °C for 72 h, afforded the diesterified products 38 and 39 in 68% and 69% yields, respectively, with both the primary hydroxyl groups esterified (Scheme 8).⁶¹

Using the above approach, polyglycerol-based dendritic amphiphiles were developed that have well-defined molecular structures possessing controlled glycine arrays on their surfaces (Fig. 3).⁶² The structure–activity relationship with respect to siRNA complexation, toxicity, and transfection profiles was studied with synthesized amphiphilic polycations. These studies revealed that a second generation amphiphilic dendrimer (G2-octaamine, 43), which has eight amine groups on its surface and a hydrophobic C-18 alkyl chain at the core of the dendron, acts as an efficient vector to deliver siRNA and achieve potent gene silencing as shown by the knockdown of luciferase and GAPDH gene activity in HeLa cells.⁶² Interestingly, the amphiphilic vector is nontoxic even at a higher ratio of N/P 100 (the ratio of moles of the amine groups of the cationic vector to those of the phosphate ions of DNA/RNA). To the best of our understanding, this was the first example of successful *in vitro* siRNA transfection using dendritic amphiphiles. We believe that this dendritic complex may potentially serve as a new promising substitute for nonviral siRNA delivery systems.

4. Biocatalytic synthesis of PEGylated block copolymers

One of the keys to the emerging advanced technologies over the past decade has been the use of nanomaterials. The aggressive advances in the fields of smart materials, solid state devices and biomimetic technologies, and the concurrent push towards miniaturization are making the understanding and development of materials at the nanometer level critical and encouraging. The design of nanoscale structures and functionalities into material systems is developing rapidly. The focus on nano-structuring of material systems has been further sharpened by the need to develop materials having novel and/or enhanced properties without resorting to new synthetic chemistries that have associated environmental and economic issues. Polymeric nanoassemblies, such as micelles of various morphologies, toroidal assembled polymersomes, nanofibers and macroscopic tubes have attracted great attention. These organized materials ranging from the nano to micro to macro scale have found broad applications in several areas, such as bioengineering, biomedicine, cosmetics, materials science and pharmaceuticals.^{63–71} In the last two decades, there has been significant growth in the area of enzyme catalyzed

polymer synthesis for a wide variety of applications and it has been the subject of various reviews in the past.^{72–74} In particular, lipase catalyzed condensation polymerizations and ring-opening polymerizations have resulted in the synthesis of unique polymers for biomedical applications. However, a large number of reports in this area utilized commonly available monomers or building blocks, which in turn restricted the post-functionalization to allow broader applications or the ability to fine-tune the



Scheme 8 Lipozyme catalysed transesterification of generation-1 dendrons. This includes the ring opening polymerization of cyclic lactones,⁷⁵ such as ϵ -caprolactone^{76,77} and pentadecalactone⁷⁸ using immobilized CAL B (Novozyme 435) to generate polyesters, but the lack of a handle for post-functionalization limits their applications. Efforts to polymerize functional cyclic lactones or copolymerization with other functional monomers resulted in limited success.⁷⁹ Similarly, enzyme catalyzed condensation polymerization using adipic acid, hexane diol and other bi-functional building blocks have been successfully reported,^{80,81} further progress in this area focused mainly on designing functional diols or diacids, which offers flexibility for post-modification to conjugate bio-active molecules.⁸² Our vast experience of enzymatic transformations on small molecules^{10–28,33–35,39,42–45,47–49} has allowed us to utilize these extremely efficient and selective biocatalysts on customized monomers for synthesizing polymers and polymeric amphiphiles that aggregate to form nanospheres for various applications. Our efforts primarily focused on synthesizing polyethylene glycol (PEG) based block copolymers considering the biocompatibility of these polymers for biomedical applications.

4.1 Biocatalytic synthesis of PEG-based drug delivery systems

Targeted and controlled drug delivery utilizing carrier systems prepared with a wide range of synthetic and natural polymers is currently a very active area of research. This is due to their low half-life, high clearance rate and non-specific actions of the

administered drugs inside the blood stream. One of the objectives of targeted and controlled drug delivery is to reduce the amount of drug administered and control its non-selective distribution by using targeting ligands that keep the therapeutic potential high. Among the many nanoparticulate pharmaceutical carriers being studied for the delivery of extremely hydrophobic drugs, amphiphilic architectures are of particular interest and have significantly affected drug delivery research.^{83,84} Our efforts in this field successfully employed Novozym 435 (CAL B) in synthesizing PEG based amphiphilic polyesters and polyamides block co-polymers utilizing various linkers.^{84,85} These polymers have been studied for their capability to encapsulate drugs, dyes and natural products. *Candida antarctica* lipase efficiently catalyzed the polycondensation of dimethyl 5-hydroxyisophthalate and polyethylene glycol of varying sizes in a solvent-free system. The molecular weight of the polyethylene glycol used strongly affected the course of the polymerization reaction. In addition, the molecular weight of the polymer formed increased under reduced pressure and at higher temperatures.^{86,87} The present reaction system afforded a variety of biodegradable amphiphilic polymers via non-toxic enzymatic catalysis under mild reaction conditions without the use of organic solvents. Furthermore, the biocatalytic approach offers the opportunity of post-polymerization modification of the copolymer, which is not feasible using conventional means. By adopting this methodology, we have synthesized a series of amphiphilic copolymers 44

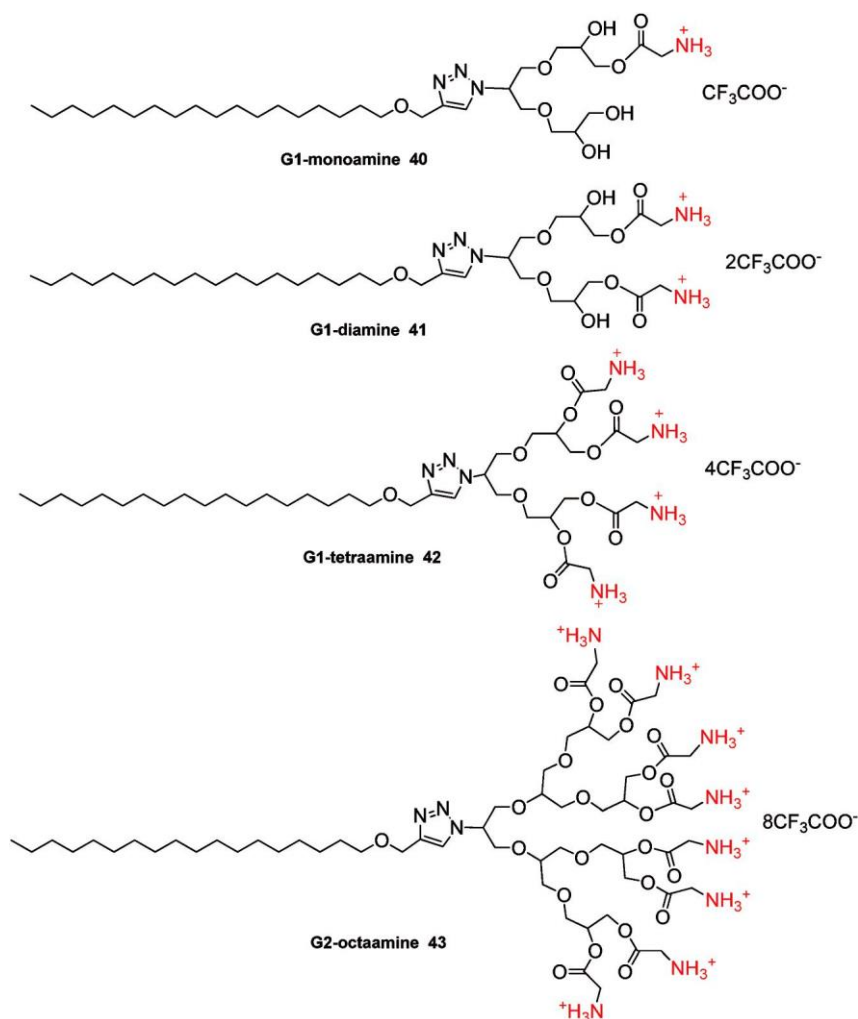


Fig. 3 Structures of the PG-amphiphiles investigated.

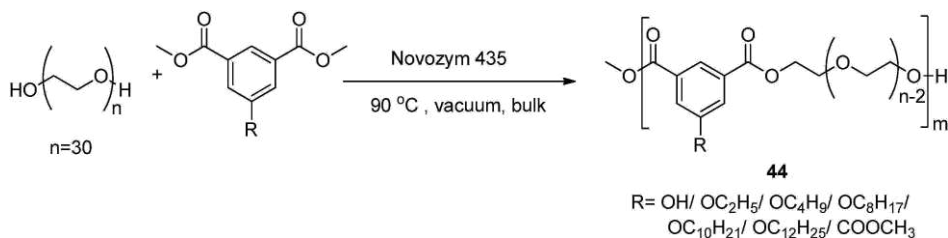
(Scheme 9) by simply attaching different alkoxy or acyloxy groups at the C-5 hydroxyl group of the polymer. Even the polymers using 5-amino isophthalate were synthesised. It has been observed that the polymer that lacks any hydrophobic substituent linked to the C-5 amino/hydroxyl group is unable

to aggregate in an ordered manner, implying the need for a hydrophobic group at this position.^{84–88}

The amphiphilic polymers 44 aggregate in aqueous medium forming nanospheres of sizes 20–35 nm and act as potential drug delivery agents as they can encapsulate small hydrophobic drugs.⁸⁸

The influence of EDA–p interactions was also studied in the drug encapsulation ability of the nanospheres formed by the copolymers.⁸⁹ To demonstrate the potential of the nanospheres formed by the amphiphilic polymers 45–47 as drug carriers, a detailed investigation was conducted on the capability of the nanospheres formed by 45 to load the anti-inflammatory agents, aspirin and naproxen (Fig. 4).^{88,89}

In vivo studies done by encapsulating anti-inflammatory agents (aspirin and naproxen) in these polymeric nanomicelles and applying them topically showed a significant reduction in



Scheme 9 Lipase catalyzed copolymerization of PEG and C-5-substituted isophthalates.

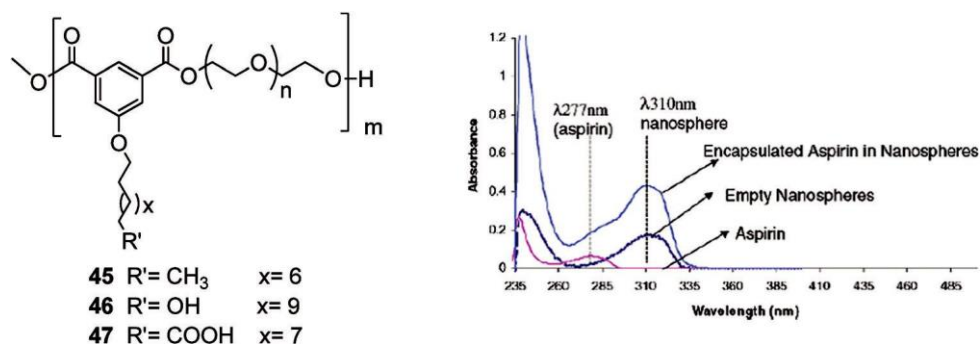


Fig. 4 UV absorption spectra of (a) aspirin, (b) empty nanosphere and (c) aspirin in polymeric nanosphere 45.

inflammation in a mice model.⁸⁸ The polymers synthesized from 5-aminoisophthalate possessing an aminoacyl chain demonstrated better drug loading capacity for a wide variety of bioactive compounds than the previously reported polymers carrying acyloxy chains (Scheme 10).⁹⁰

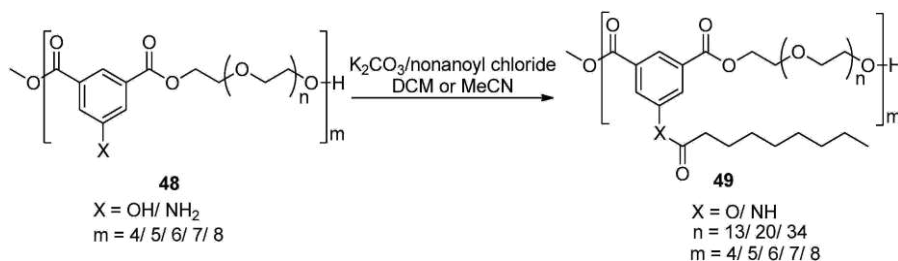
The copolymers 50–56 were evaluated for their drug encapsulation capacity using curcumin, a bio-active naturally occurring compound of current interest in chemical and biological research worldwide. The synthesized polymers having long alkyl chains attached via an ether linkage, viz. 50–53 demonstrated a better curcumin loading capacity than the identical polymers having a long acyl chain attached via an ester linkage, viz. 54–56. Particle sizes were found to be in the range of 20–50 nm for polymers 50–54 and 56, whereas polymer 55 showed a particle size of ca. 88 nm (Fig. 5). There was no significant change in the particle size of these amphiphilic polymers after encapsulation of curcumin, except for polymer 50.⁹¹

The selective interactions of the phenolic moiety of our PEGylated polymers with alkali and alkaline earth metal ions

were investigated, and Ca²⁺ ions were found to preferentially bind to the co-polymers 57a–c constituted by the phenols and PEG (Fig. 6).⁹² The advantage of using the PEG unit is that it confers solubility to these aromatic polyester polymers (57a–c and 58–60) in aqueous medium. These polymeric systems can be used for selective detection and estimation of Ca²⁺ ions in the presence of other cations. This may have promising applications in the field of analytical biochemistry.⁹²

The guanidine-based polymeric system 61 (Fig. 7) has been developed with the aim that these cationic polymers will increase the cellular uptake and may prove to be useful for gene delivery as they can undergo interactions with the negatively charged phosphate groups of the DNA/RNA/cell surface.⁹³

In addition to the copolymerization approaches discussed above, terpolymerization reactions have also been performed by some research groups to synthesise novel drug delivery carriers using poly(ethylene glycol) (PEG), sebacic acid (SA) and 1,3-bis-(carboxyphenoxy)propane (CPP) as the monomers.⁹⁴ The synthesis of a novel class of terpolymers was accomplished by us through



Scheme 10 Functionalization of 5-hydroxy and 5-amino groups of the polymer 48.

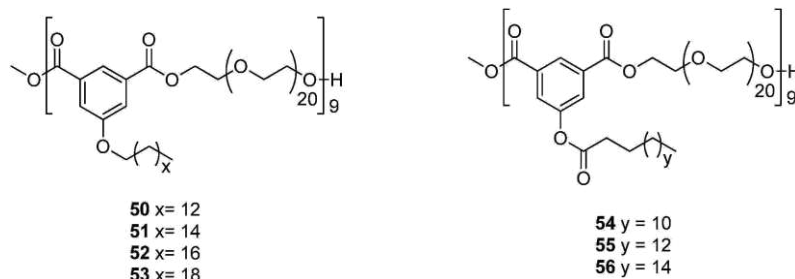


Fig. 5 Functionalized amphiphilic polymers.

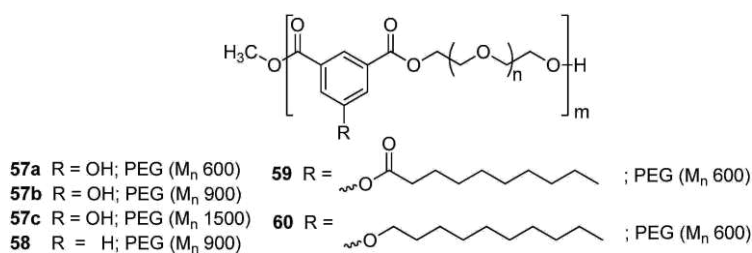


Fig. 6 Enzymatically catalyzed polymers for the selective Ca^{2+} ion binding studies.

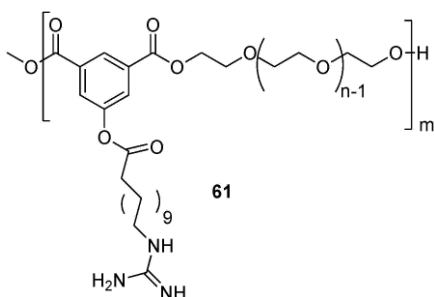


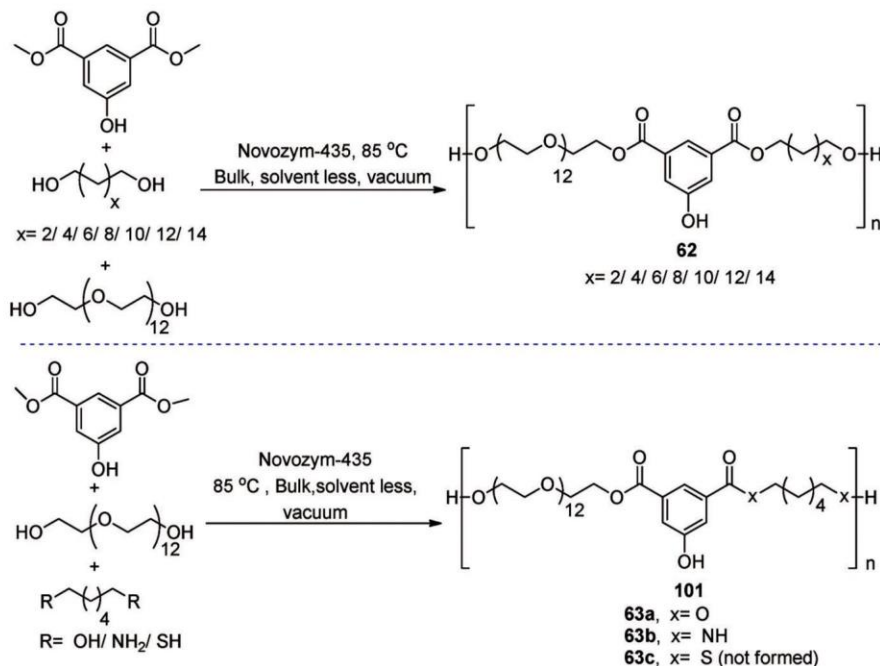
Fig. 7 Guanidine-functionalized copolymers.

incorporation of a third component during the copolymerization of dimethyl 5-hydroxyisophthalate with poly(ethylene glycol) 600 under solventless conditions using Novozym-435.⁹⁵ These multi-component polyesters and mixed polymers with polyester and polyamide linkages have aromatic as well as aliphatic moieties incorporated into their backbone. The properties of these terpolymers were compared and studied vis-a-vis the addition of the third component having different functionalities, i.e. compounds 1,6-hexanediol, 1,6-hexanediamine or 1,6-hexanedithiol

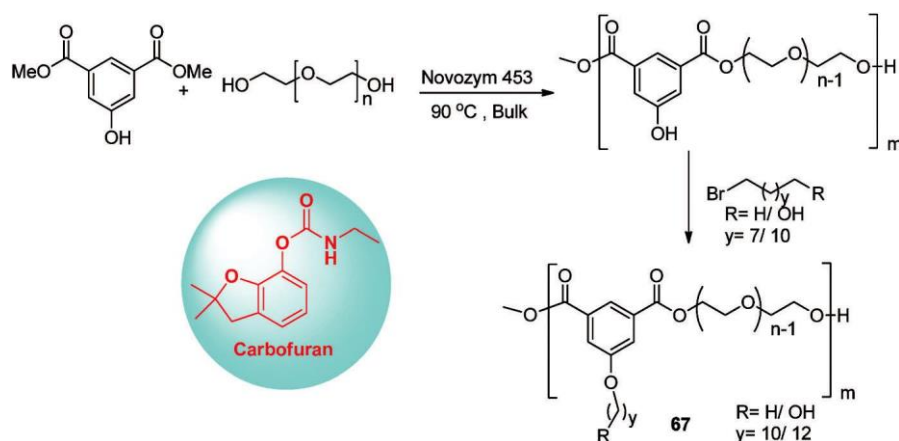
during terpolymerization reaction of dimethyl 5-hydroxyisophthalate with polyethylene glycol (Scheme 11).⁹⁵

Vitamin E is known for its ability to capture the energetic free radicals (reactive forms of oxygen) and therefore to help combat their potential skin damaging effects. Unfortunately, it is difficult for the vitamin E molecule to penetrate and spread through the outer layers of skin on its own. We have designed and synthesized novel nano-carriers for incorporating vitamin E (Fig. 8).^{96,97} These nanocarriers with covalently linked vitamin E moieties have enhanced capacity to encapsulate vitamin E. Such systems are unique due to the presence of both bound as well as encapsulated vitamin E, and this allows a better control over the release and bioavailability of vitamin E. The amphiphilic nature of these nano-carriers make them soluble in both hydrophilic and lipophilic media.^{96,97}

The vitamin E conjugated copolymers 64 are highly soluble in water but do not aggregate to form nano-micelles as observed using a static light scattering technique. This may be due to the improper balance of hydrophilic to hydrophobic segments. Therefore, the partially (30%) vitamin E conjugated copolymers 64 were further reacted with excess of bromodecane to completely



Scheme 11 Chemo-enzymatic multi-component copolymerization.



Scheme 12 Synthesis of copolymers 67 used for carbofuran encapsulation.

aqueous media and were used for the encapsulation of carbo-furan (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate), an insecticide-nematicide. Thus, a controlled release formulation (Scheme 12) has been developed, perhaps for the first time, where amphiphilic nanopolymers have been used for the development of a controlled release pesticide application.⁹⁹

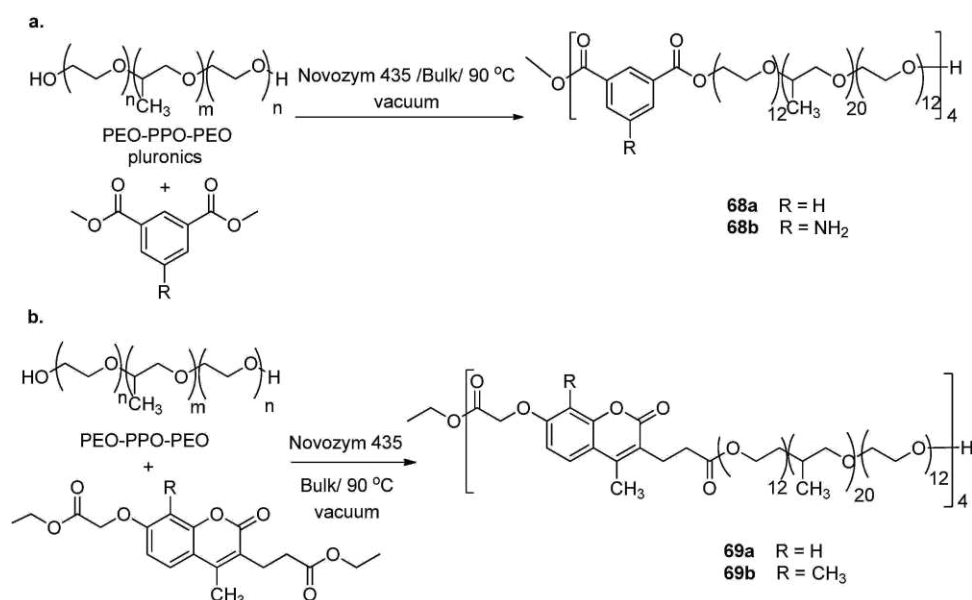
4.2 Biocatalytic synthesis of pluronic based polymers

The poly(ethylene oxide)–poly(propylene oxide) (PEO–PPO–PEO) triblock copolymers family of pluronics, with different numbers of hydrophilic ethylene oxide and hydrophobic propylene oxide units, have an adequate hydrophilic–lipophilic balance. Due to their amphiphilic character, these copolymers display surfactant properties including an ability to interact with hydrophobic surfaces and biological membranes. We have synthesized amphi-philic pluronic-based copolymers using CAL-B.¹⁰⁰ The developed

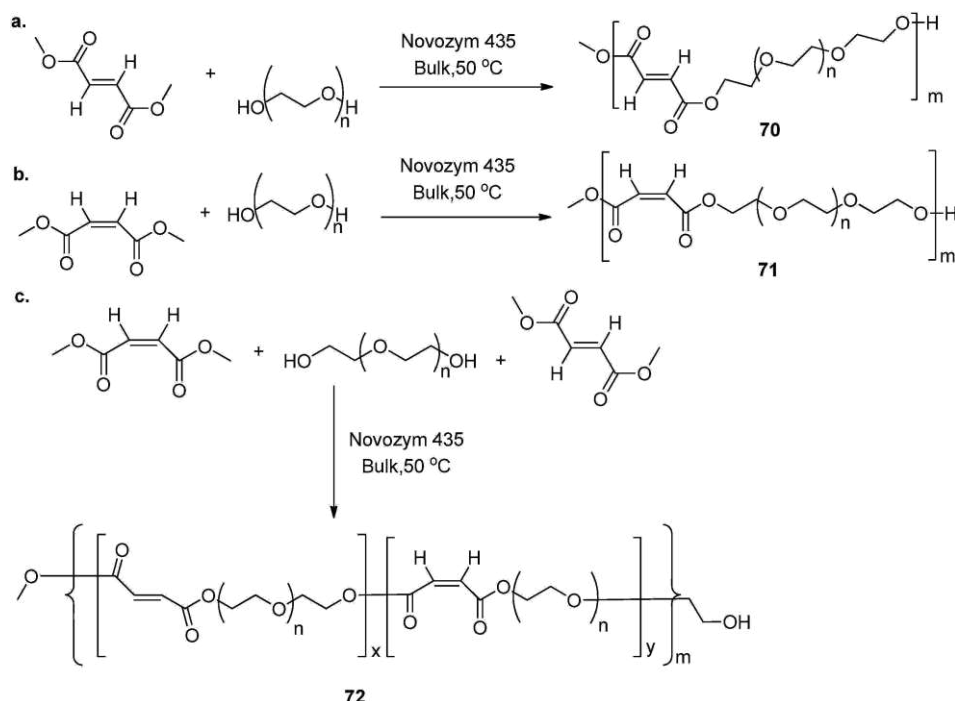
polymeric materials 68 and 69 were found to be capable of solubilizing/encapsulating the hydrophobic drug curcumin (Scheme 13). The percentage solubilization/encapsulation of curcumin was found to be in the range of 2.7–5.7% with respect to the weight of the different polymers used. The percentage encapsulation of curcumin was estimated using UV spectroscopy in methanol.¹⁰⁰

4.3 Biocatalytic synthesis of PEG dimethyl fumarate and dimethyl maleate based polymers

An enzymatic copolymerization of PEG with dimethyl fumarate and dimethyl maleate under solventless conditions provided the corresponding copolymers 70 and 71, respectively (Scheme 14).¹⁰¹ Though no cis/trans isomerization was observed under enzymatic polymerization conditions, the saturation of double bonds remains a problem similar to that observed in the chemocatalyzed polymerization of these esters. A terpolymerization of dimethyl



Scheme 13 Biocatalytic synthesis of pluronic based polymers.



Scheme 14 (a) Copolymerization of PEG-dimethyl fumarate. (b) Copolymerization of PEG-dimethyl maleate. (c) Terpolymerization.

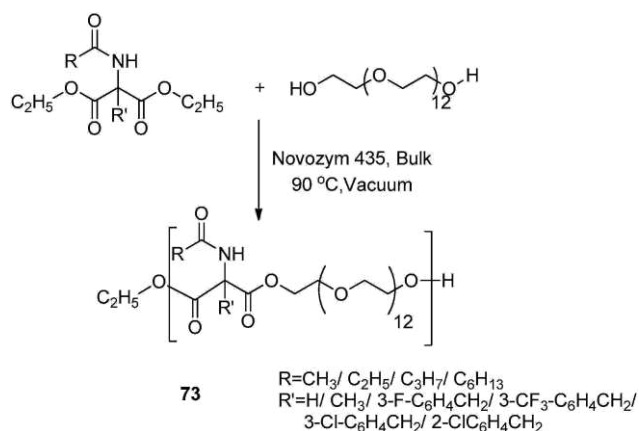
fumarate, dimethyl maleate and PEG was also carried out to give the terpolymers **72**.¹⁰¹

4.4 Biocatalytic synthesis of copolymers of PEG and non-proteinogenic amino acid derivatives

Amino acids, the building blocks of all proteins, are biochemically involved in every biological process and thus make them essential for human life. They are non-toxic, biocompatible and economical. Their high cellular permeability and fixed stereochemistry prompted us to use them as building blocks for polymer synthesis.¹⁰² Also the great versatility and the inherent high affinities of peptides for their respective targets have led to tremendous progress for their therapeutic application in the last few years. In order to enhance their utility in therapeutic applications, their chemical modifications are of great interest. One of the approaches to modulate their pharmacokinetic properties is the frequently used PEGylation methodology.¹⁰³ In light of the above, we have carried out lipase catalyzed con-densation copolymerization of a number of non-proteinogenic amino acid derivatives and polyethylene glycol (PEG, MW 600) in bulk (Scheme 15).¹⁰²

4.5 Biocatalytic synthesis of glycerol-PEG based copolymers

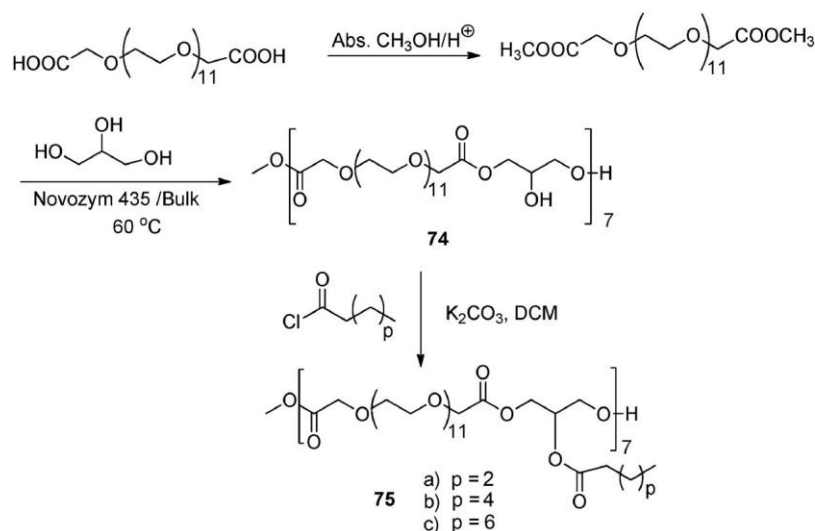
Glycerol is one of the most versatile and valuable chemical substances and is utilized in a variety of commercial products with no known adverse pharmacological or environmental effects. Moreover, it exhibits good chemical stability and inertness in biological systems. We have designed and developed the Novozym 435 catalyzed biocatalytic method to synthesize novel polymeric systems **74** using glycerol and poly[ethylene glycol bis(carboxymethyl)ether] dimethyl ester.¹⁰⁴ Both the synthons



Scheme 15 Biocatalytic synthesis of PEG and non-proteinogenic amino acid copolymers.

used in the co-polymer synthesis are biocompatible, non-toxic and are readily available. The polymerization proceeds regio-selectively through the primary hydroxyl groups of glycerol leaving the secondary hydroxyls free for post-coupling with saturated fatty acid esters, or possibly coupling with drugs/bioactive molecules. Amphiphilic polymers **75** were synthesised by attaching alkyl chains at the secondary hydroxyl groups of these PEG-glycerol copolymers simply by acylation (Scheme 16).¹⁰⁴

The synthesised amphiphilic polymers **75a-c** show aggregation under aqueous conditions, thus forming nanoparticles in the range of ca. 51.7–57.1 nm size and the critical micelle concentration (CMC) value of the polymer **75c** in aqueous media was determined using a static light scattering technique, and was found to be 0.024 M. The supra-molecular organization



Scheme 16 Lipase catalysed synthesis of glycerol-PEG based copolymers.

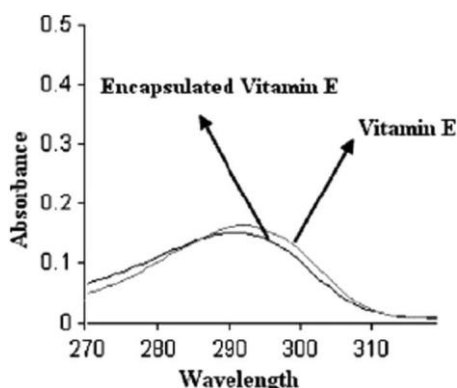
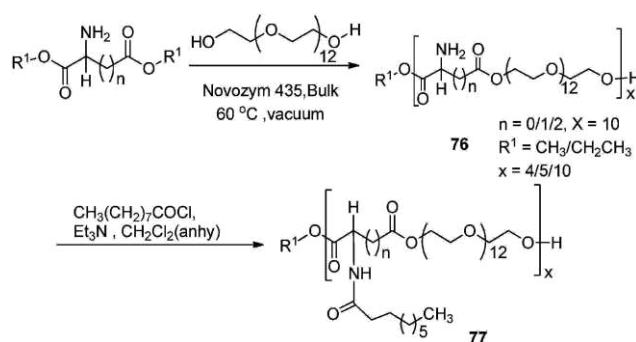


Fig. 10 UV absorption spectra of an encapsulated hydrophobic drug vitamin E in the copolymer 75c using methanol as a solvent.



Scheme 17 Chemoenzymatic synthesis of PEG and amino acid diester based copolymers.

of polymers 75a–c in aqueous and organic media was studied using static light scattering. The drug encapsulation capability of these polymeric systems was evaluated by attempting the encapsulation of hydrophobic vitamin E, the percent-age encapsulation of vitamin E was found to be quite satisfactory viz. 22% with respect to the weight of the polymer (Fig. 10).¹⁰⁴

4.6 Biocatalytic synthesis of PEG and amino acid diester based copolymers

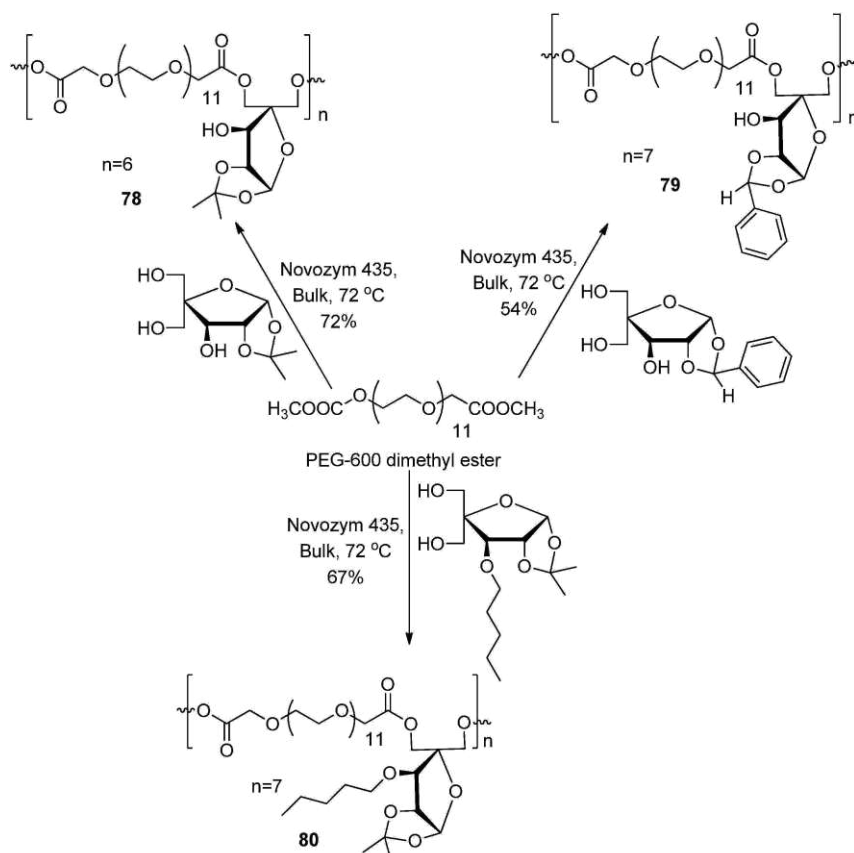
To synthesize amphiphilic copolymers based on amino acid diesters and PEG, a chemo-, regioselective enzymatic method-ology was designed. The condensation polymerization was catalyzed by immobilized *Candida antarctica* lipase (Novozym 435) under solventless conditions. The free amino group after copolymerization was functionalized with acyl chloride (Scheme 17). The availability of a free amino group after

copolymerization is a significant achievement in terms of polymer therapeutics.¹⁰⁵

4.7 Biocatalytic synthesis of sugar-PEG based copolymers

Novel sugar-PEG-based polymers 78–80 were synthesized by the enzymatic copolymerization of 4-C-hydroxymethyl-1,2-O-isopropylidene-b-L-threo-pentofuranose/4-C-hydroxymethyl-1,2-O-benzylidene-b-L-threo-pentofuranose/4-C-hydroxymethyl-1,2-O-isopropylidene-3-O-pentyl-b-L-threo-pentofuranose with PEG-600 dimethyl ester using Novozyme-435 (Scheme 18).¹⁰⁶

Carbohydrate monomers were obtained through a multi-step synthesis starting from D-glucose diacetone, and PEG-600 dimethyl ester was obtained by the esterification of the commercially available PEG-600 diacid. Aggregation studies on the copolymers 78–80 revealed that in aqueous solution, those polymers bearing the hydrophobic pentyl/ benzylidene moiety, viz. 79,80, spontaneously self-assemble into supramolecular aggregates. The polymeric aggregates were further explored for their drug encapsulation properties in buffered aqueous solution of pH 7.4 (37 1C) using Nile red

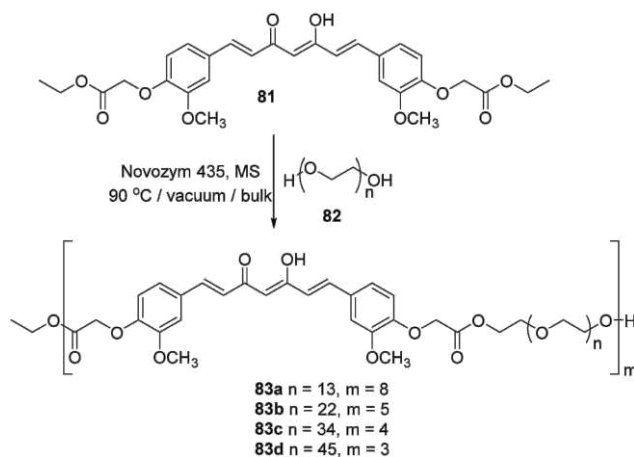


Scheme 18 Biocatalytic synthesis of Sugar-PEG based copolymers.

as a hydrophobic model compound by means of UV/Vis and fluorescence spectroscopy.¹⁰⁶

4.8 Biocatalytic synthesis of PEGylated curcumin block copolymers

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a central transcription factor that regulates the antioxidant defense system and is considered as a modifier for several inflammatory diseases. Thus, activation of Nrf2 provides a pivotal therapeutic target for developing therapies against these diseases. Herein, a chemo-enzymatic methodology has been designed and developed to make PEGylated curcumin derivatives as water soluble drug candidates with enhanced aqueous solubility and bioavailability. For this, the curcumin diester **81** was copolymerized with poly(ethylene glycol) (**82**) using Novozym 435 under solventless conditions (Scheme 19).¹⁰⁷ Novozym 435 promotes selective trans-esterification and only catalyses the reaction of the primary hydroxyls of poly(ethylene glycol). It does not affect the secondary enolic hydroxyls of curcumin, thus leaving the active moiety in curcumin unaltered. A luciferase based reporter gene assay was used during primary screening for establishing the anti-inflammatory activity of this novel Nrf2 activator. Most of the PEGylated curcumin analogs **83a–d** strongly activate Nrf2 several folds higher than the free curcumin but copolymer **83a** was identified as the most potent Nrf2 activator.



Scheme 19 Synthesis of PEGylated curcumin block copolymers.

Copolymer **83a** induces Nrf2-driven NQO1 expression in a concentration dependent manner.¹⁰⁷

5. Biocatalytic synthesis of photoresponsive amphiphilic polymers

Photochemical reactions, which occur in small molecules, can also be induced to occur in macromolecules. Though in

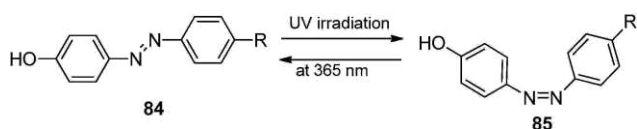


Fig. 11 Photochemical reaction in the cis–trans isomerism of an azobenzene.

macromolecular environments, there are constraints which are not present in the small molecules, the challenge is to apply fundamental principles to macromolecules which may coil, branch, or be chemically cross linked increasing the order of complexity. Likewise, molecular mobility plays an important role in determining the course of photochemical behaviour in polymers and is related to the size of the molecule, the flexibility of the polymer chain, and whether the polymer is in solution or in the solid state.

We have designed and synthesized photo responsive amphiphilic polymers using a novel chemo-enzymatic methodology to study their supra-molecular organization in aqueous and organic media so that their supramolecular organization can be changed in a controlled manner.¹⁰⁸

Azobenzene-containing polymers have attracted considerable attention in the last decade due to their potential applications as photoactive compounds for molecular devices, information storage systems and photochemical switching (Fig. 11). Keeping this in mind, the copolymer 86 was synthesized through the *Candida antarctica* lipase B (Novozym 435) catalyzed condensation polymerization of dimethyl 5-hydroxy isophthalate with polyethylene glycol as discussed earlier, and alkylated with azo benzenes 87–90.¹⁰⁸ The resulting polymers 91–94 were studied for their cis/trans isomerization behavior upon UV irradiation (Scheme 20).

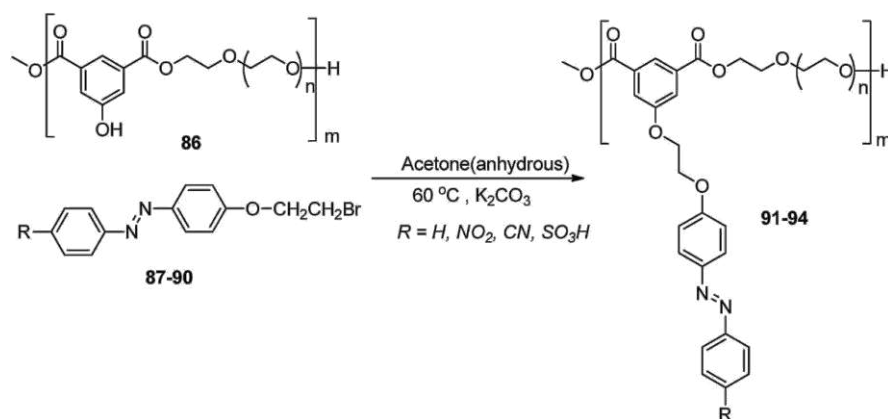
Progress was also made in the development of a novel chemo-enzymatic approach for the covalent attachment of photoresponsive units into the RNA backbone.¹⁰⁹ This involved lipase catalyzed acylation of the C-2⁰ hydroxyl group in the ribose sugars in the RNA molecule to incorporate photo-isomerizable azobenzene groups into the RNA strands (Scheme 21). A reverse micellar approach was used for this RNA functionalization to maintain the solubility of the nucleic acid as well as to limit the preferred

hydrolysis reaction in aqueous media. The azobenzene groups incorporated into the RNA molecule show photo-isomerization behavior and can serve as optical ‘handles’ for the manipulation of the conformation of RNA. The photoactive group incorporated into the RNA molecule opens new avenues for the investigation of RNA conformational switching through the photodynamic behavior of the azo chromophore as well as for the fabrication of versatile functional biomacromolecular matrices.¹⁰⁹

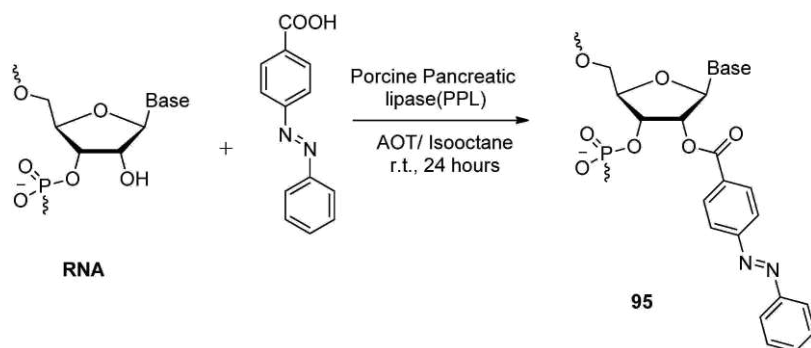
In another report, photoresponsive polymeric amphiphiles 96a–b (Fig. 12) have been developed by first synthesizing the polyester chain via Novozym 435 (CAL B) catalyzed condensation polymerization of poly[ethylene glycol bis(carboxymethyl)ether]-diethylester and 2-azidopropan-1,3-diol. The resulting polymers 96a–b aggregated in aqueous media to form supramolecular micellar aggregates as shown by fluorescence measurements using ‘Nile red’ as a probe. The nano-structures formed in the aqueous solution were studied using dynamic light scattering and cryo-TEM measurements. The encapsulation potential of poly-meric amphiphiles for Nile red and curcumin as well as their release via cis–trans photoisomerization of the embedded azobenzene moiety was studied. The developed polymeric micellar systems behave as efficient photoresponsive smart nanocarriers.¹¹⁰

6. Polymeric electrolytes for nanocrystalline solar cells

Dye-sensitized solar cell (DSSC)-devices based on molecular dyes are reported to be capable of injecting electrons into the conduction band of oxide semiconductors in their photo excited state, and are currently attracting wide interest due to their potential for converting solar light into electricity at a low cost. A variety of tethered PEGylated polymers were developed using a mild, environmentally benign and highly selective biocatalytic approach for preparing a quasi-solid electrolyte system for dye-sensitized solar cells (DSSCs). The biocatalytic approach was based on Novozyme-435 catalyzed copolymerization reactions; the polymeric materials obtained were further function-alized with hydrophobic and hydrophilic side chains using mild chemical reactions (Scheme 22),¹¹¹ and the resulting polymeric



Scheme 20 Synthesis of photoresponsive amphiphilic polymers 91–94.



Scheme 21 Synthesis of azo functionalized RNA.

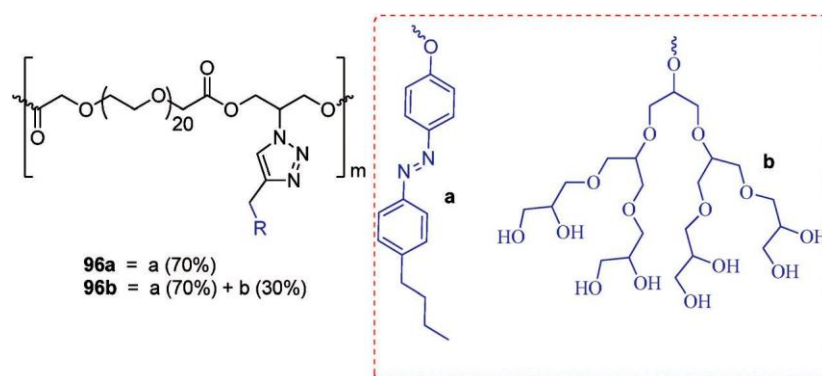


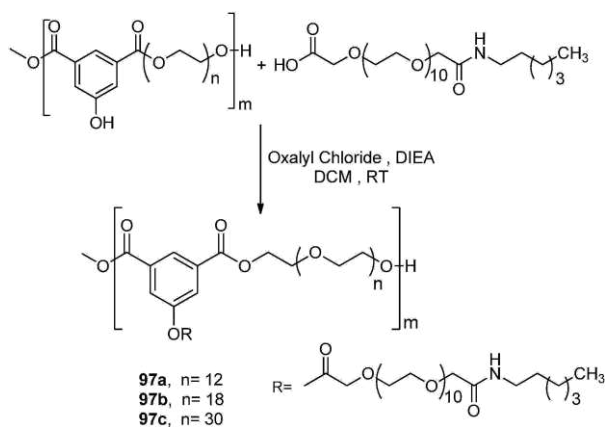
Fig. 12 Synthesis of an azobenzene functionalized polymer and its dendronized analogue.

materials were used in formulating quasi-solid electrolyte compositions and incorporated into flexible DSSCs. Quasi-solid electrolytes prepared from biocatalytically synthesized polymers 97a–c (Scheme 22) by adding at least 25% of the polymer in an ionic-liquid-based electrolyte composition showed a PV efficiency of 4.30%. Almost identical results (PV 4.45%) were observed from the cells containing an ionic-liquid-based electrolyte that did not contain the polymers 97a–c, thus indicating that the presence of polymers 97a–c has no adverse effect on the conversion efficiency. In contrast, it increases the stability of the devices as it forms a gel

and does not contain any volatile solvent. The measured ionic conductivity of the formulation used in these devices is 2×10^{-5} Siemens(S) per cm.¹¹²

The ionic conductivity is not an intrinsic property of the PEG polymer but depends on the details of the formulation used. The results from photovoltaic characterization studies on polymers 97a–c based gel electrolytes incorporated into flexible solar cells show that the photovoltaic (PV) performance is similar, irrespective of the viscosity of the polymer electrolyte at similar polymer concentrations. This is a very promising result, as the viscosity of the gel can be altered without affecting the PV performance of the solar cells. The intensity-dependent PV characterization study suggests that maximum PV performance is achieved at an intensity level of around 0.5 Sun (50 mW cm^{-2}). An efficiency of over 4.6% was achieved for the polymer 97c based gel-incorporated flexible cell at 55 mW cm^{-2} , which is about 10% higher than under the 1 Sun condition.^{111,113}

The ion transport properties and photovoltaic performance of polymers 98a–d (Fig. 13) – based on quasi-solid electrolytes incorporated photo voltaic cells – have also been studied. The polymeric electrolytes 98a–d were synthesized through the Novozym-435 catalyzed copolymerization of dimethyl 5-hydroxyisophthalate and poly(ethylene) glycol of various sizes as described earlier. These polymers were incorporated into Konarka's redox electrolyte formulations and were applied onto dye sensitized titanium oxide coated plastic substrates. After applying the electrolyte, the dye-sensitized titanium oxide



Scheme 22 Chemoenzymatic synthesis of PEGylated polymer 97a–c.

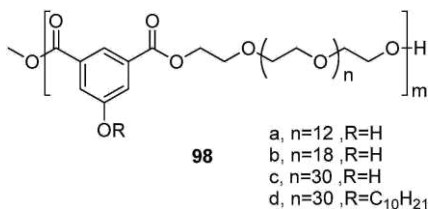


Fig. 13 Enzymatically synthesized polymeric electrolyte.

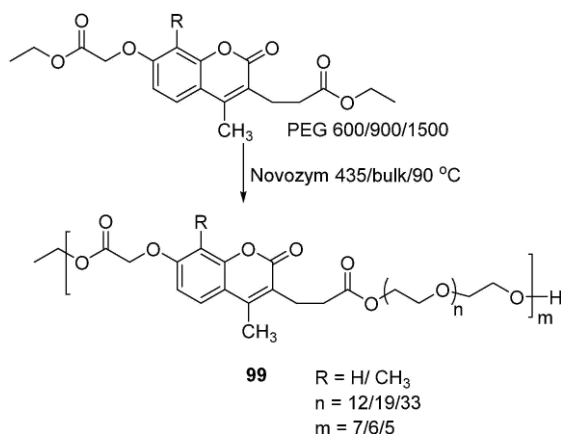
coated flexible substrate was sandwiched with a platinum coated ITO/PET substrate and heat-sealed to obtain flexible plastic cells. The fabricated cells were characterized for photo-voltaic (PV) performance using a solar simulator under AM 1.5 conditions. It was observed that the solar conversion efficiency of the solar cells depended strongly on the polymer micro-structure used in formulating the redox electrolyte and showed a photovoltaic efficiency up to 3.0%.¹¹⁴

7. Biocatalytic synthesis of PEG coumarin block copolymers

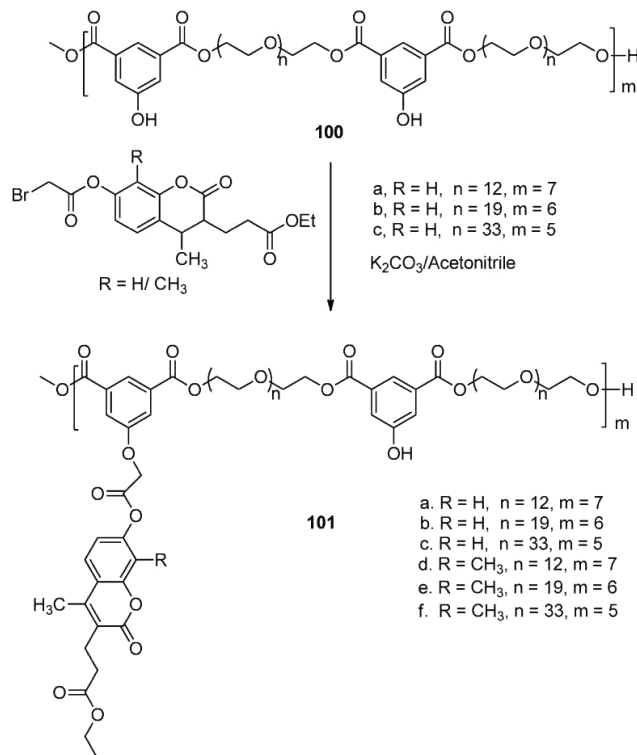
4-Methylcoumarins are well known for their antioxidant and anti-edema activities.

To increase their antioxidant potential and hydrophilicity, lipase (Novozym 435) catalyzed polymerization of C-4 methyl-coumarin diesters and polyethylene glycols (PEGs) was carried out to produce the novel copolymers 99 (Scheme 23).¹¹⁵ These novel PEGylated C-4 methyl- and C-4, C-8 dimethylcoumarin derivatives were evaluated for their ability to lower ICAM-1 (intercellular cell adhesion molecule-1, whose overexpression leads to inflammation) expression.^{115,116}

In another approach, the C-4 methyl- and C-4, C-8 dimethylcoumarins were attached covalently to the PEG-based copolymers 100 (obtained through CAL-B catalyzed copolymerization as discussed earlier) (Scheme 24).¹¹⁶ The synthesized derivatives were evaluated for their anti-inflammatory activities with respect to their ability to inhibit TNF- α induced ICAM-1 (intercellular cell adhesion molecule-1) expression on human endothelial cells.



Scheme 23 Synthesis of PEGylated C-4 methylcoumarins.



Scheme 24 Coupling of bromo analogues of C-4 methyl- and C-4, C-8 dimethyl coumarins with PEGylated polymers.

It was observed that the PEGylated 4-methyl- and 4, 8-dimethylcoumarin derivatives were more effective than their non-PEGylated analogues in inhibiting ICAM-1 expression. The PEGylated 4-methyl- and 4,8-dimethylcoumarins have shown an enhanced and improved ability to inhibit TNF- α induced ICAM-1 expression on human endothelial cells as compared to the monomeric analogues. The additional advantage of synthesized PEGylated co-polymers is their enhanced solubility in aqueous and organic media, thus making them suitable for bio-medical preparations and applications.¹¹⁶

Coumarins comprise a very large class of phenolic compounds and are well known for their optical properties. Copolymers were synthesized using PEG and polydimethyl siloxane (PDMS) containing coumarins for the detection of DNT and TNT (Fig. 14). The fluorescence quenching of these co-polymers in solution can be attributed to the collisional quenching. The fluorescence quenching of 50 nm-thick films of these co-polymers is 60% and 20% due to DNT and TNT vapours, respectively, at room temperature. The significant quenching of fluorescence for 50 nm-thick films is attributed to the low glass transition temperature of PEG and PDMS which provides pathways for analyte diffusion even at room temperature and consequently sensitivity is not limited to the analyte diffusion. This approach is simple but unique and could be extended to other chromo-phores sensitive to the detection of explosives. The response of these polymeric sensors 102 and 103 is promising, and they can easily detect DNT and TNT at the level of a few parts per billion.¹¹⁷

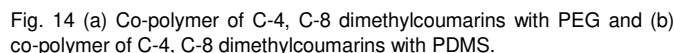
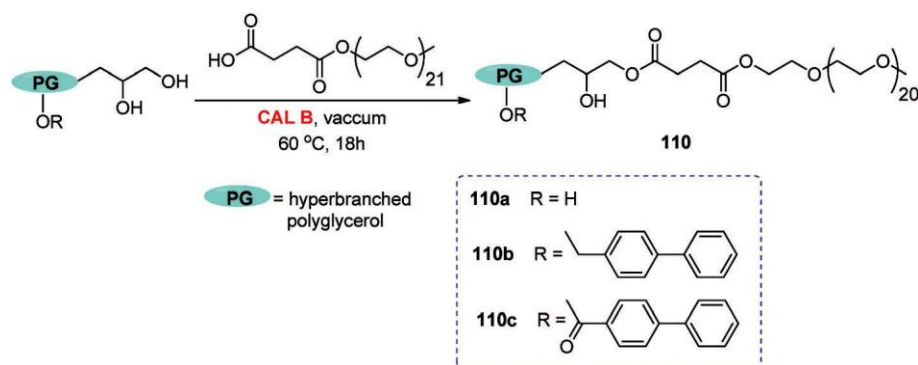


Fig. 16 Controlled synthesis of the PG-PEG nanoparticles for dermal drug delivery applications.



Scheme 25 Bifunctional nanocarrier systems based on amphiphilic hyperbranched polyglycerol (hPG).

hydrophobic interactions as well as p-p stacking, analogous to a unimolecular micelle system. A second guest molecule, which has a high affinity for the shell, like Nile red, was solubilized in the outer shell of the host molecule, thus connecting the nanocarrier molecules forming aggregates. This model is confirmed using UV-Vis, fluorescence, atomic force microscopy, and dynamic light scattering, as well as release studies triggered by pH-changes and enzymes. Different control release profiles were observed for the encapsulated guest molecules in the core and in the shell. The bifunctional nanocarrier system is a promising carrier system for concurrent delivery of more than one hydrophobic drug for combination therapies.¹²⁰

The biocatalytic synthetic approach has also been utilized for the creation of a new class of non-ionic dendronized multi-amphiphilic polymers and their self-assembly to micellar nanocarriers has been studied. The polymers were constructed from biocompatible PEG chains and PG dendrons to form a hydrophilic outer shell and alkyl chains molding for a hydrophobic interior. The polymer backbone was synthesized from linear PEG and 2-azido-1,3-propanediol using a biocatalytic pathway. The versatility of this basic structure originates from its azido groups, which facilitate an easy functionalization by Click grafting with appropriate alkynes. As a proof of concept, the polymeric backbone was grafted with polyglycerol dendrons of different generations and octadecyl chains in varying proportions, and this led to a series of new multi-amphiphilic polymers 117a-f that self-assemble to generate well-defined, globular micelles with diameters in the range of 10 nm at low critical aggregation concentrations (10^{-5} M) as was shown by the DLS, cryo-TEM and surface tension measurements (Fig. 17). The potential ability of these micelles to function as nanocarriers and as prospective drug delivery systems was illustrated with the hydrophobic model dye pyrene.¹²¹

In another study, the synthesis of PEG-1000-diethyl ester and 2-azido-propane-1,3-diol-based copolymers has been achieved utilizing Novozym 435. The linear base copolymer was then functionalized with polyglycerol-based regular [G1.0] and [G2.0] dendrons and C12/C14 hydrophobic alkyl chains via an efficient Click Chemistry approach (Scheme 26). The resulting amphiphilic dendronized polymers 118a-d form well-defined

micelles in aqueous solutions. The transport behavior of these polymers was studied by using Nile red as a fluorescent model dye. The cytotoxicity profiles of a few representative polymers were evaluated, and the effect of hydrophobic alkyl chain length as well as hydrophilic polyglycerol dendron's generation on the biocompatibility of the polymeric systems was studied.¹²²

To gain further insight into the factors that affect stability and transport efficiency under dilution conditions, dendronized and hyperbranched multifunctional amphiphilic polymers were synthesized by following the "grafting to" approach using varied amounts of propargylated alkyl chain with perfect and hyperbranched polyglycerol dendrons on the base copolymer of PEG (M_n : 1000 g mol⁻¹) diethylester and 2-azidopropane-1,3-diol following the biocatalytic method and Click Chemistry approach. The dendronized and hyperbranched polymeric systems form supramolecular aggregates and exhibit an efficient transport potential for the model dye "Nile red" in the low mM range in the core-shell-type architecture provided with distinct amphiphilicity as required for encapsulation (Scheme 27).¹²³ Cytotoxicity studies show the polymeric systems to be non-toxic over a wide concentration range. The cellular internalization of Nile-red-encapsulated supramolecular micellar structures was also studied using cellular fluorescence microscopy and fluorescence-activated cell sorting (FACS) measurements. A comparison of the data for the dendronized polymers (PEG M_n : 600/1000 g mol⁻¹) with the respective low-molecular weight amphiphiles reveal that these polymeric systems are excellent nanotransporters.¹²³

In another approach, a new class of amphiphilic molecular transporters has been developed through the Novozym 435 catalyzed copolymerization of PEG diethyl ester and azido-triglycerol via its primary hydroxyl groups. The co-polymer was further grafted by using the C12 alkyl chain and polyglycerol dendron moieties via the azide group of the polymer backbone by following the 'Click' chemistry approach (Fig. 18). The secondary hydroxyl groups on the backbone provide an additional site for acylation and fine-tuning the physico-chemical properties. The encapsulation potential of these polymeric architectures was explored for Cy3 dye. Although most of the amphiphilic polymers form stable micelles, polymer 120a behaved differently in terms of

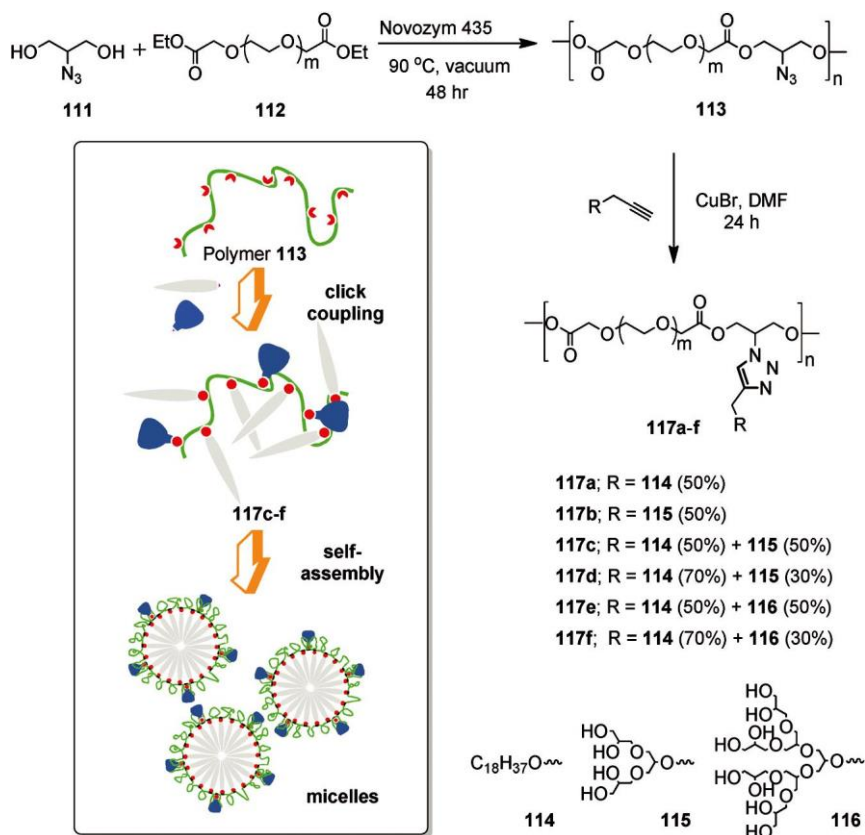
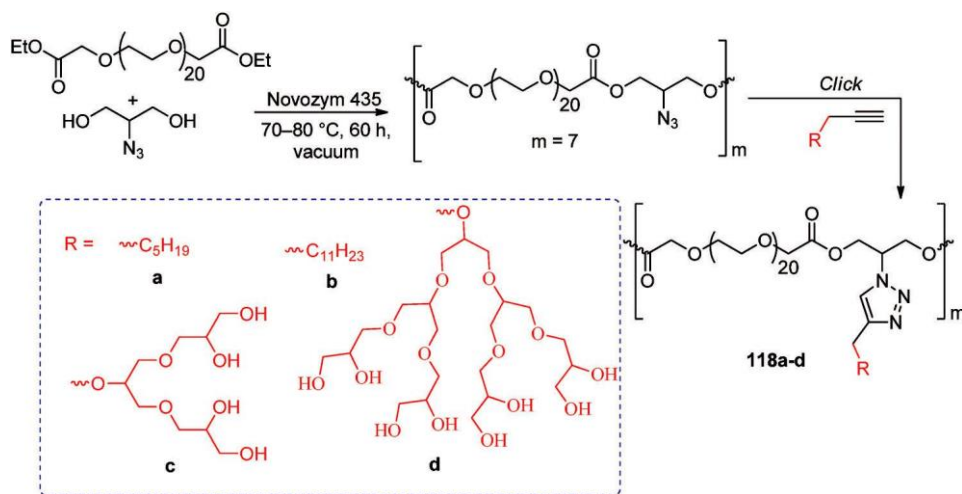


Fig. 17 Biocatalytic synthesis of dendronized multi-amphiphilic polymers.

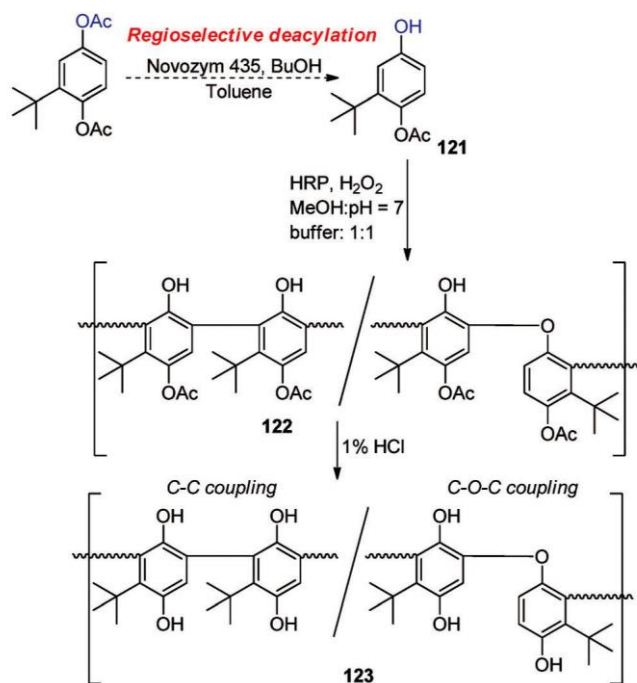


Scheme 26 Synthesis of multifunctional amphiphilic dendronized polymers 118a-d.

aggregation as it exhibits the formation of vesicles along with micelles. However, all the polymers show a significant Cy3 transport capacity in the range of 7.5–19.1 mmol mol⁻¹ of polymer. The cellular internalization and in vitro study of dendronized polymers by means of CLSM measurements revealed that the [G2.0] grafted polymer 120c exhibits better transport results.¹²⁴

9. Biocatalytic synthesis of novel polymeric antioxidants

Many industrial products such as plastics, elastomers, lubricants, petroleum-based products, cooking oil, cosmetics, as well as processed food products undergo oxidative, mechanical, thermal and photo degradation. The primary cause of degradation has



Scheme 28 Biocatalytic synthesis of poly(t-BHQ).

10. Biocatalytic approach for developing novel organosilicon polymers as flame retardants (FRs)

Flame retardants (FRs) comprise a diverse group of chemicals which are widely used at relatively high concentrations and find many applications, including in the manufacture of electronic equipments, textiles, plastics, polymers and in the aviation and automobile industries.¹⁴² The global annual consumption of flame retardants is currently over 1.5 million tons with a market value of over 6 billion dollars. The use of FRs is primarily to protect materials against ignition and to limit (or prevent) fire related damages. More than 175 different types of FRs exist and are commonly divided into four major groups: inorganic FRs, organophosphorus FRs, nitrogen containing FRs and halogenated organic FRs.¹⁴³ The mode of incorporation into the polymeric material divides the FRs into two additional classes, namely reactive or additive. Reactive FRs are chemically bonded into plastics, i.e. HET acid, TBBPA, DBNPG or other organophosphorus compounds. Additive FRs are numerous and more frequently used. They are blended with the polymers and thus are more likely to leach out of the products, e.g. HBCD,

aluminium trihydrate, magnesium hydroxide and phosphate esters.^{144,145} Currently, because of their high performance efficiency and low cost, the largest group of FRs are the brominated FRs. Typical conventional polymers using phenolic compounds as monomers are phenol formaldehyde-resins. These polymers are widely used in various fields, such as wood composites, fiber bonding, laminates, foundry resins, abrasives, friction and molding materials, coatings and adhesives, and flame retardants. Environmental concerns over the potential

risks that halogenated chemicals pose have been around for decades. These are rooted in the persistence, bioaccumulation and toxicity (PBT) associated with specific brominated organic compounds.¹⁴⁵ To respond to this, the FR research community and others began developing non-halogenated flame retardants.

The great synthetic flexibility of organosilicon polymers, viz. their ease of processing, low cost, and non-toxic nature, presents an attractive alternative solution over current flame retardant materials.¹⁴⁶ Siloxane-based hybrid polymers that have organic and inorganic components have been synthesized using enzymatic methods under mild and solventless conditions.^{147–149} A number of aromatic siloxane-based new polyesters and polyamides 124a–b were prepared and evaluated for their flame retardant properties. In addition, siloxane-based aliphatic polyamides 125 were also synthesized (Fig. 19).

Flame retardant and thermal properties of the biocatalytically generated copolymers 124a–b and 125a–c were studied at their onset decomposition temperatures. The copolymers 124a and 124b were found to start losing weight around 400 °C, while this temperature drops to around 290 °C for the copolymers 125a, 125b and 125c. Thus, copolymers 124a and 124b are relatively heat-resistant and can be used up to fairly high temperatures. It was observed that the copolymers 124a and 124b exhibit flammability similar to Kevlar or poly(ether etherketone) (PEEK), the two commercial products of Dupont. For applications requiring ultra-fire-safe polymers, it is preferable to use polymers whose heat-release capacity is 100 J g⁻¹ K⁻¹ and under, such as polyamides 125b and 125c.

Furthermore, nanoclay was used as a potential additive in the above siloxane copolymers, which was then blended with polyolefin materials to improve their thermal as well as flame retardant properties. Moreover, the addition of siloxane co-polymer 126 (Fig. 20) in polypropylene improved the thermal stability of polypropylene by 60 °C, whereas the improvement in thermal stability of polypropylene went up by 73 °C when polypropylene was blended with nanoclay mixed siloxane co-polymer 126. Thus, on the basis of decomposition temperatures, polypropylene blended with a mixture of nanoclay and siloxane co-polymer 126 was found to be relatively more heat-resistant and can therefore be used at fairly high temperatures.

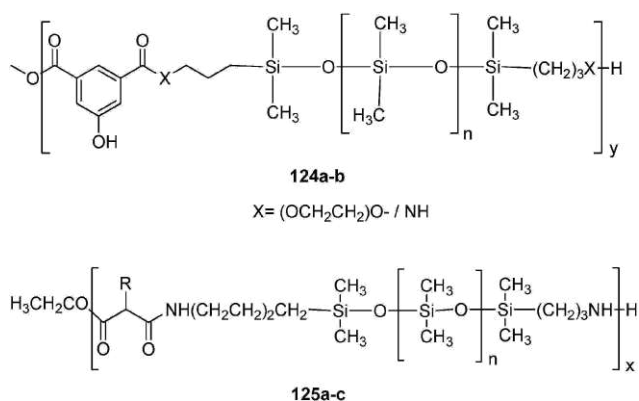


Fig. 19 Silicone-based aromatic/aliphatic polyamides and polyesters.

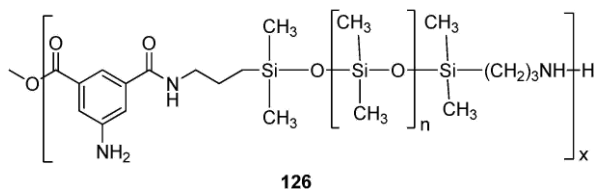


Fig. 20 Silicone-based 5-aminoisophthalate polyamides.

The mixtures of nanoclay and siloxane co-polymer 126 showed a better performance in terms of heat release capacity as compared to siloxane co-polymer 126 alone in polypropylene, and a decrease of $68 \text{ J g}^{-1} \text{ K}^{-1}$ in heat release capacity was observed from polypropylene to polypropylene blended with nanoclay and the siloxane co-polymer 126.^{148,149}

To enhance the efficacy of the biocatalytically synthesized copolymers 124–126, their nanocomposites containing titanium dioxide (TiO_2) were prepared, and TiO_2 nanoparticles were also prepared for comparison. The thermal and flame-retardant properties of these materials were investigated. Thermogravimetric analysis (TGA) studies were carried out on various TiO_2 –polymer compositions to investigate the effect of TiO_2 nanoparticles on the decomposition temperature of these novel polymers. The decomposition temperature of polymer 127a

is increased from $408 \text{ }^\circ\text{C}$ to $425 \text{ }^\circ\text{C}$ by adding 20 wt% of nc- TiO_2 (Fig. 21).¹⁵⁰

Another study shows how the use of chemoselective enzymatic synthesis of polydimethylsiloxanes, based upon a 5-aminoisophthalate moiety, increases the aromatic stabilizing groups in polydimethylsiloxane and also creates functionality available for cross linking (Scheme 29).

Hexamethylene tetramine (HMTA) is a common phenolic cross linker, its cross linking capacity and ability decreases the flammability of the polymer by removing the branched siloxane pathway to degradation, giving rise to only the more stable cyclic siloxane structures.¹⁵¹ The copolymer 128, crosslinked with various concentrations of hexamethylenetetramine (HMTA), gave the crosslinked polymeric system 129, and the heat release capacity decreased with the increase in wt% of HMTA. In the crosslinked copolymer 129 with 20 wt% of HMTA, the HR capacity drops to $90 \text{ J g}^{-1} \text{ K}^{-1}$, which is very promising and an improvement over the commercially available Dupont's flame-retardant polymers, Kevlar ($292 \text{ J g}^{-1} \text{ K}^{-1}$) and PEEK ($180 \text{ J g}^{-1} \text{ K}^{-1}$). However, the char yields and total heat release remained practically similar in all the HMTA and copolymer 129 compositions.¹⁵¹

Enzymatic copolymerization of polydimethylsiloxanes carrying amino end groups with diesters of C-4-methylcoumarins,

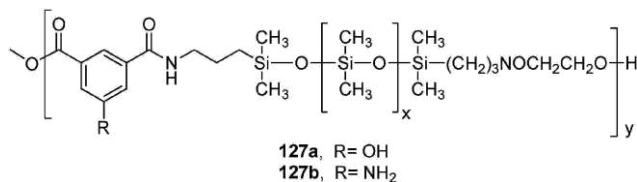
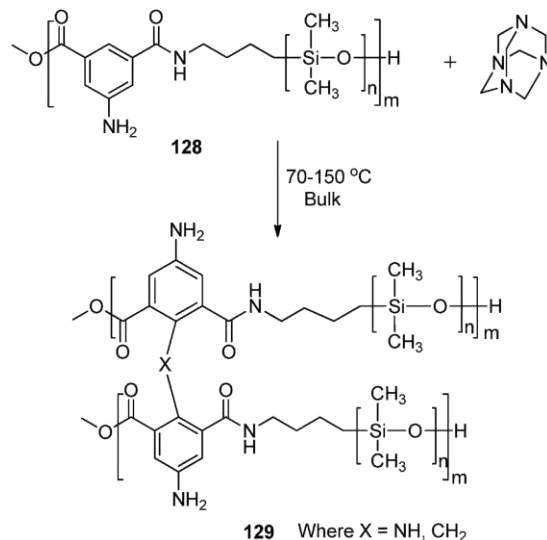


Fig. 21 Enzymatically synthesized siloxane copolyamides.



Scheme 29 Synthesis of novel crosslinked polydimethyl siloxanes.

using the lipase, *Candida antarctica*, as a biocatalyst, has been carried out to yield novel polymers 130 that have C-4-methylcoumarin moieties in the backbone.^{152,153} In a separate synthesis, C-4-methylcoumarin was also incorporated as a pendant moiety into the poly siloxaneisophthalate copolymers 131 by functionalization of the hydroxyl groups in the isophthalate moiety (Fig. 22).^{152,153}

Recently, another novel approach of cross linking of enzymatically synthesized polydimethyl siloxane copolymers 132 with aromatic dianhydrides was also reported by us (Scheme 30).^{152–154} The resultant cross linked siloxane copolymer 133, could easily be coated (in solution form) onto any substrate including fabrics and cross linked on the surface by heat treatment. This work has provided rigid, aromatic and thermally stable cross linkers for further improvement of the flame retardant properties of the materials in comparison to siloxane copolyamides

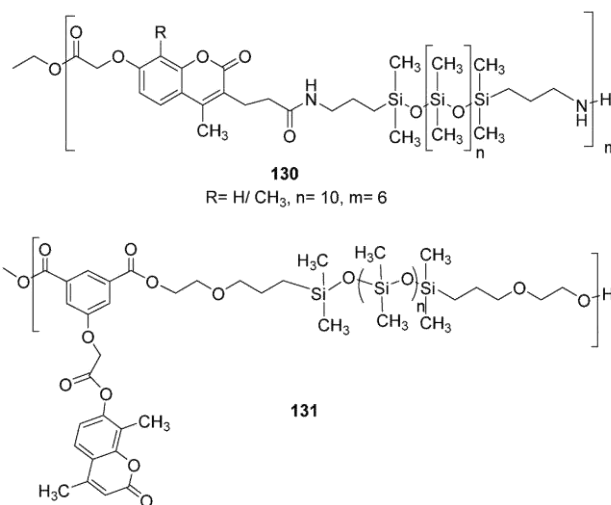
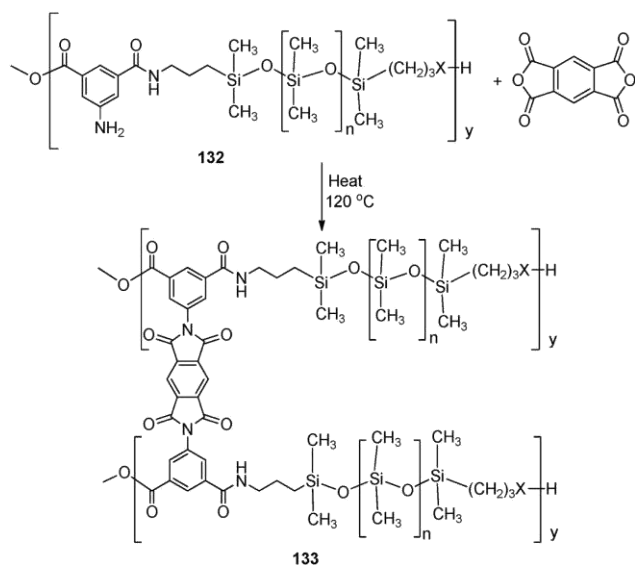


Fig. 22 Coumarin-based siloxane polymers.

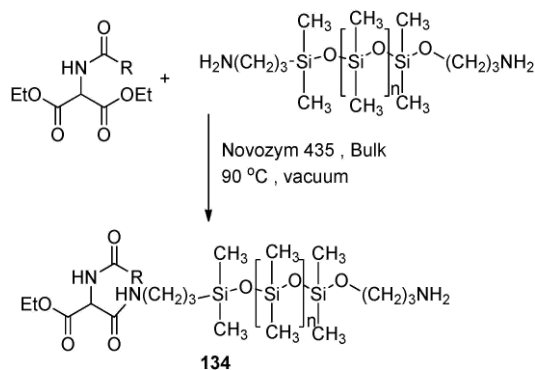


Scheme 30 Synthesis of polydimethyl siloxane copolymers with aromatic dianhydrides.

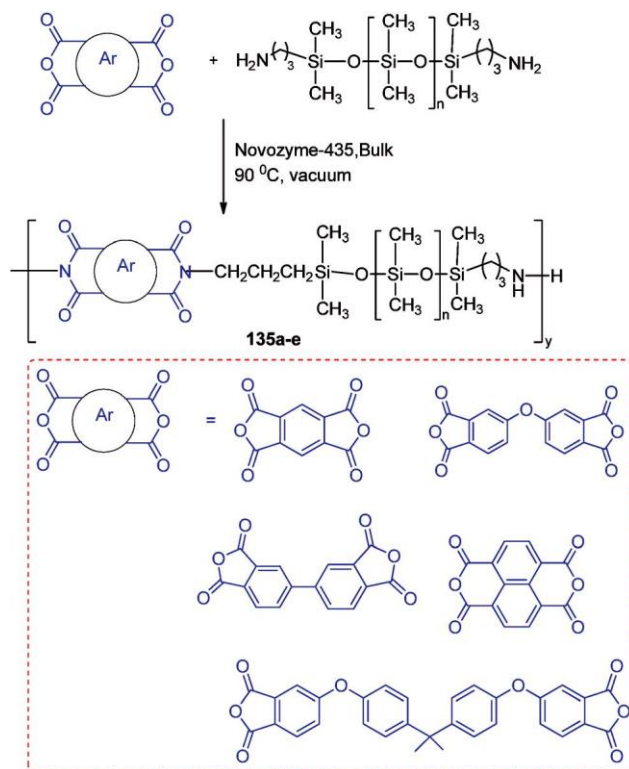
when they were cross linked using hexamethylenetetramine (HMTA) as the cross linking agent.^{152–154}

As discussed earlier, the condensation co-polymerization of non-proteinogenic amino acid derivatives with polyethylene glycol (PEG) was developed (Scheme 15) for a controlled release system in which the drug is physically adsorbed or entrapped within the polymeric matrix. We have investigated the flame retardant properties of the hybrid copolymers 134, formed by the bio-catalytic acylation of non-proteinogenic amino acid diesters by amino terminated polysiloxanes of varying molecular weights (600 and 900) in bulk (siloxane acting as solvent also) (Scheme 31). The degree of polymerization was extremely dependent on the nature (hydrophilic/hydrophobic) of the macromer and the length of the alkyl chain in the malonate derivatives as well as on the nucleophilicity of the reactive functional group of the macromer.¹⁵⁵

The first biocatalytic synthesis of siloxane copolyimides, siloximide E (135), using aminopropyl-terminated polydimethylsiloxane and 4,4'-oxydiphthalic anhydride in the presence of



Scheme 31 Biocatalytic acylation of diethyl malonate derivatives with diaminosiloxanes.



Scheme 32 Siloxane-based polyimides.

lipase as the biocatalyst without using any solvent was performed by us (Scheme 32).¹⁵⁶

To confirm the role and establish the importance of using lipase as a catalyst in these reactions to drive the reaction to imide formation, the biocatalytically synthesized polyimide was compared with the same polyimide synthesized without using a biocatalyst. It is known in the literature that polyimide preparation using traditional chemical methods (without a biocatalyst) involve the initial formation of polyamic acid, followed by ring closure to form the polyimide. It is interesting to note that in our enzymatic synthesis, the cyclization takes place in situ and gives rise to a clean linear polyimide product without the formation of polyamic acid, thus avoiding the need for any further processing and purification.

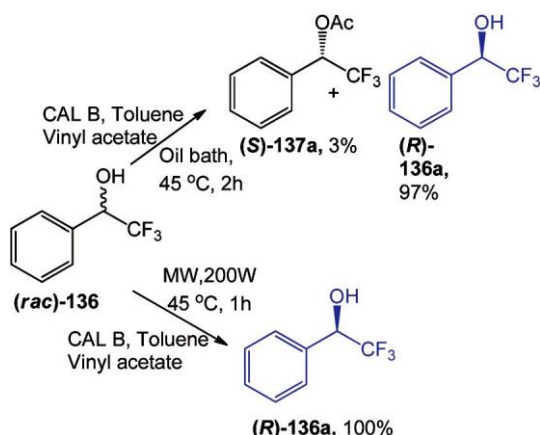
11. Unconventional media for lipase catalysis

11.1 Microwave-assisted lipase catalyzed synthesis

Microwave-assisted synthesis is well-known in organic and radiochemistry for milder reaction conditions, enhanced rates of reactions and a reduction in overall reaction times.^{157–163}

However, its applications in enzyme catalyzed reactions are fairly new. This section vignettes a few examples of microwave-assisted heating in lipase catalyzed reactions and their impact on reaction time, reaction conditions, product yield, chemo-, regio- and enantioselectivity in comparison to conventional heating.^{164–167}

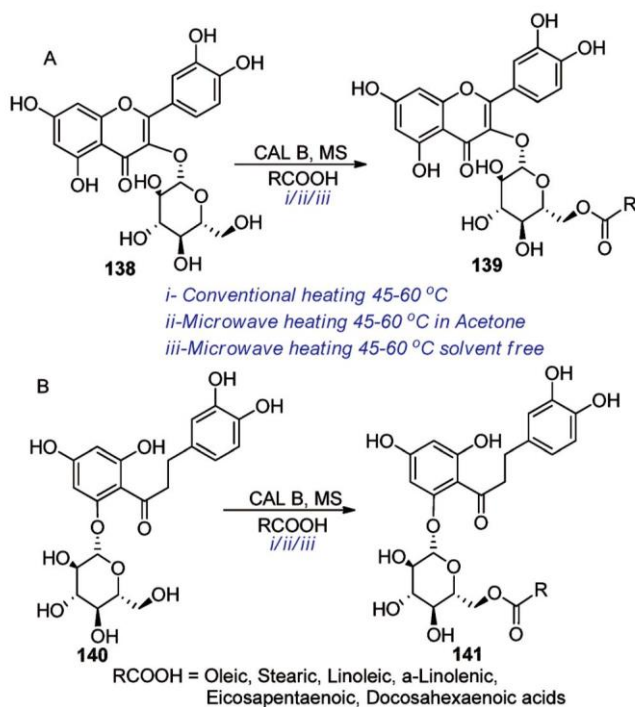
Ribeiro et al. attempted a lipase (CAL B) catalyzed enantioselective acetylation of (S)-2,2,2-trifluoro-1-phenylethanol



Scheme 33 Comparison of CAL B catalyzed acetylation under micro-wave and convention heating.

(136) with vinyl acetate under conventional heating using an oil bath and microwave heating (Scheme 33).¹⁶³ Interestingly, under microwave irradiation the racemic mixture was resolved to 100% of the R-isomer of 2,2,2-trifluoro-1-phenylethanol (136a) but no acetylation was observed.¹⁶⁵ Whereas, under conventional oil bath heating, 3% of the (S)-isomer of the acetylated analog 137a was observed along with 97% of the R-isomer of 2,2,2-trifluoro-1-phenylethanol in 2 h (136a).

In another study, Ziaullah et al. investigated the effect of microwave heating on CAL B catalyzed esterification of quercetin-3-O-glucoside and phloretin-2⁰-glucoside with a variety of long chain fatty acids (Scheme 34) in comparison to conventional



Scheme 34 CAL B catalyzed esterification of quercetin-3-O-glucoside and phloretin-2⁰-glucoside under convention and microwave heating.

heating under similar molar ratios of reactants and enzyme.¹⁶⁴ In conventional heating, lipase catalyzed esterification of 138 and 140 to 139 and 141, respectively, were achieved between 18–24 h with 81–98% yield.¹⁶⁴ Whereas, when the same reactions were carried out under microwave heating, esterified products were obtained only in 120–160 seconds with a similar molar quantity of the reactants and enzyme in slightly better yields. Moreover, when the authors carried out the same esterification reactions under solvent-free conditions under microwave heating, the reaction time was even further reduced to 75–105 seconds with 85–98% yields.¹⁶⁴ The present study evidently demonstrates faster lipase catalyzed esterification under microwave heating with a drastic reduction in the overall reaction time compared to conventional heating.

11.2 Ultrasound-assisted lipase catalyzed synthesis

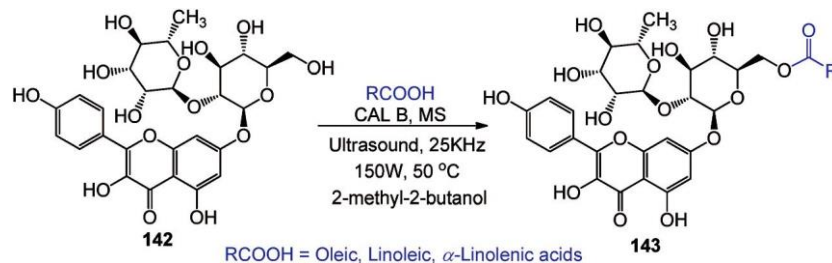
Similar to microwave-assisted lipase catalyzed synthesis, studies have been done to evaluate the effect of ultrasound on lipase catalyzed esterification reactions.^{166–169} Zheng et al. have investigated lipase catalyzed esterification of naringin (142), a well-known flavonoid with various unsaturated fatty acids after ultrasound pretreatment to 143 (Scheme 35).¹⁶⁸ In this study, the authors have reported that an ultrasound pretreatment of the reaction mixture has accelerated the rate of reaction and reduced the overall reaction time from 96 h to 72 h with a considerable enhancement in ester 143 yield in comparison to ultrasound untreated reaction.¹⁶⁸

11.3 Lipase catalyzed reactions in ionic liquids

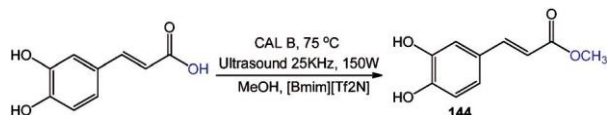
In another study, Wang et al. observed an increase in the rate of the reaction when lipase-catalyzed synthesis of methyl caffeate (144) was carried out under ultrasound irradiation in an ionic liquid.¹⁶⁹ In this study, the lipase catalyzed esterification of caffeic acid with methanol using 25 kHz ultrasound frequency at 150 W power in a [Bmim][Tf₂N] ionic liquid at 75 °C was carried out. The reaction was completed in 9 h with a 99.79% yield of methyl caffeate.¹⁶⁹ However, in a conventional incubator shaker, the same reaction was completed in 36 h with a 96.54% yield. This study clearly demonstrates enhancement in the rate of reaction and a 4-fold reduction in reaction time with a somewhat increase in the yield of methyl caffeate (144) (Scheme 36).¹⁶⁹

11.4 Lipase and ruthenium catalyzed dynamic kinetic resolution of secondary alcohols

Kinetic resolution is the differentiation of two enantiomers from a racemic mixture and is based on the difference in the rates of reaction of an individual enantiomer over the other with a chiral catalyst or reagent.^{170–175} During this process, one of the two enantiomers remains unreactive and as a result a maximum of 50% of the theoretical yield can be achieved.^{173,174} However, this limitation can be addressed by one of the following: (i) use of a prochiral substrate,¹⁷⁰ (ii) stereoinversion of the left-over enantiomer,¹⁷¹ or (iii) dynamic kinetic resolution.^{172–175} In the case of dynamic kinetic resolution, the substrate continuously undergoes isomerization/racemization by virtue of another catalyst



Scheme 35 CAL B catalyzed esterification of naringin under ultrasound pretreatment.



Scheme 36 CAL B catalyzed synthesis of methyl caffeate in an ionic liquid under ultrasound irradiation.

even during the process of kinetic resolution with a chiral catalyst and thus all the racemic mixture can be utilized and even up to 100% theoretical yield can be achieved.

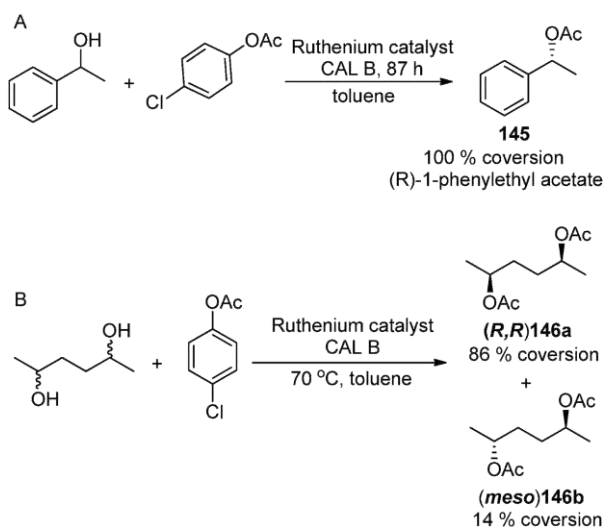
Persson et al. applied a combination of ruthenium metal complex $[\text{Ru}_2(\text{CO})_4(\text{m-H})(\text{C}_4\text{Ph}_4\text{COHOCC}_4\text{Ph}_4)]$ for substrate racemization and *Candida antarctica* lipase for enantioselective acylation (Scheme 37) in the same reaction for an efficient and high yield kinetic resolution.¹⁷³ In this study, the authors investigated two different ruthenium complexes ($[\text{Ru}_2(\text{CO})_4(\text{m-H})(\text{C}_4\text{Ph}_4\text{COHOCC}_4\text{Ph}_4)]$) and $[(\text{PPh}_3)_3\text{RuCl}_2]$ for the dynamic race-mization of 1-phenyl ethanol and various acyl donors for enantioselective acylation using lipase to (R)-1-phenylethyl acetate (145). It is noted that 4-chlorophenyl acetate was the most suitable acyl donor among other studied acyl donors for the enantioselective acylation of 1-phenyl ethanol in the presence of ruthenium and lipase catalysts. In this study the authors

apparently showed that 100% of the racemic mixture can be transformed to an enantioselective product.

In another study, Persson et al. investigated the same combination of lipase and ruthenium complex for the dynamic kinetic resolution and acylation of secondary symmetrical diols.¹⁷⁴ A representative example of this study is depicted in Scheme 37B, where (dl/meso B 50/50) 2,5-hexanediol was treated with a ruthenium catalyst, *Candida antarctica* lipase, and with 4-chlorophenyl acetate as the acyl donor in toluene at 70 °C. The obtained product contains 86% of (R,R)-2,5-diacetoxy-hexane (146a) and 14% of (meso)-2,5-diacetoxyhexane (146b). Later Huerta et al. also applied this methodology on α-hydroxy esters for dynamic kinetic resolution and trans-esterification using the ruthenium catalyst and *Pseudomonas cepacia* lipase to obtain an enantioselective product in a decent yield.¹⁷⁵

12. Conclusions and future outlook

From the foregoing reports, it is clear that biocatalysis using lipases is a major contributor to current research and development activities to benefit a wide range of application areas. Biocatalysts, especially lipases, certainly bring a new dimension to the organic reactions and processes, and demonstrate “Green Chemistry” pathways involving fewer by-products and less (or no) toxic chemicals or reagents. This is highly useful for biomedical research where the purity of the material is of utmost importance. The amphiphilic polymers developed using the lipase mediated biocatalytic approach are biocompatible, non-immunogenic and have the required size for the glomerular filtration (renal clearance), which make them suitable potential candidates for drug delivery applications. The PEGylated compounds synthesized through the lipase assisted reactions discussed in this article hold great potential in maneuvering the creation and development of new materials that have a wide range of applications in the healthcare and industrial sectors. A significant number of marketed pharmaceuticals contain active pharmaceutical ingredients that are manufactured in part using biocatalysis as a key enabling technology. Though the utilization of biocatalysis is growing due to significant advances in technologies for enzyme discovery, supply, and improvement, as well as an increased focus on applications for chiral drugs, drug delivery systems and green chemistry, there still remains a lack of clarity around the quality and regulatory expectations when using biotransformations in research and manufacturing, and this



Scheme 37 Ruthenium and lipase catalyzed dynamic kinetic resolution and enantioselective acetylation of a secondary alcohol.

lack of clarity can be a barrier to the uptake and adoption of biocatalysis (Wells et al., *Org. Proc. Res. Dev.*, 2012, 16, 1986). Thus a rational, coherent and achievable strategy for the use of biocatalysis in the manufacture of small molecule active pharmaceutical ingredients (APIs) or drug delivery systems based on a scientific, risk-based approach to drug quality and patient safety is needed. Currently, there is no specific guidance for handling biocatalyst residues in the literature and enzyme residues should be treated as potential impurities in the final products. Essentially risk-based scientific arguments must be used to ensure the quality of the API and ultimately the patient. Commonly used drugs, like Atorvastatin (Lipitor), Sitagliptin (Januvia) and Pregabalin (Lyrica), are manufactured using biocatalysis during the GMP stage of their synthesis and the issue of enzyme residue is still under discussion (Wells et al., *Org. Proc. Res. Dev.*, 2012, 16, 1986). In spite of the efforts discussed herein to exploit lipases for various applications, a great deal of work is still necessary to achieve the goals of “Green and Sustainable Chemistry” which are being faced by the scientific community, both in academia and industry.

Conflict of interest

The authors declare that there is no conflict of interest for publishing this review in “Chemical Society Reviews”.

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